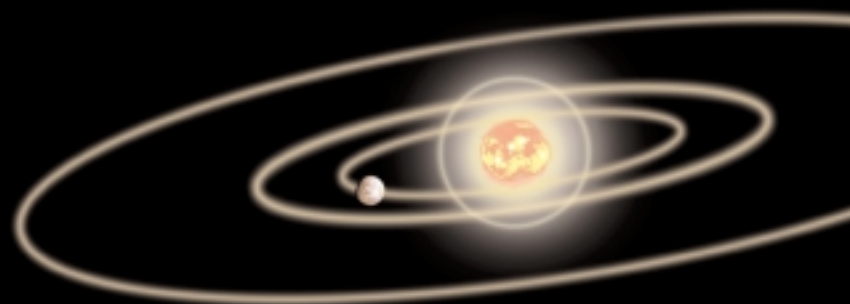


NASA ASTROBIOLOGY INSTITUTE



Annual Science Report

YEAR 2
July 1999 - June 2000

THE ANNUAL SCIENCE REPORT

This document reflects the history and beginnings of NAI (in the Director's Introduction), the current scope of research accomplishments (in the individual Lead Team reports which follow), and the nature and achievements of NAI Central. The Lead Team reports cover the period from July 1999 to June 2000, the second year of the NAI's grant awards. Each report outlines the general intent and current accomplishments of the Team's work, identifies the Team's Members, and identifies the Team's projects with specific objectives of the Astrobiology Roadmap.

PDF DOCUMENT NAVIGATION

There are several navigational options available while using this pdf document. First, the Table of Contents on page 5 has active links to each item in the table. Click on any item in the table and you will go directly to that page. Second, all of the internet url's at the beginning of each report are linked to those website's as is the NAI url you see throughout the document. Click on the url and your internet browser will open and take you directly to that site. Third, at the end of each report there is a box to click on that will take you back to the Table of Contents if you don't want to go through the report page by page.

NASA Astrobiology Institute



Annual Science Report

Year 2

July 1999 - June 2000

Annual Science Report Year 2

NASA Astrobiology Institute
Ames Research Center
MS 240-1
Moffett Field, CA 94035-1000
U.S.A.

<http://nai.arc.nasa.gov>

Baruch S. Blumberg, Director
Rosalind A. Grymes, Associate Director

Janet L. Morrison, Senior Editor
Krisstina Wilmoth, Editor
Julie Fletcher, Graphic Design & Production
Shige Abe, Editorial Support
Jeni Joiner, Editorial Support
Celeste Merryman, Editorial Support

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Baruch S.
Blumberg

The NASA Astrobiology Institute (NAI) was established to encourage and fund astrobiological research in basic science and to support the space-venturing missions of NASA. Humans have a long term interest – probably pre-dating written history – in the questions, "Are we alone in the Universe? Is life a unique event that occurred only on our own Earth, or do we exist in a life-rich Cosmos?" These, coupled with the other fundamental questions, "How did life begin?" and "What is the future of life in the Universe?" encompass the subject matter of astrobiology. In the last half decade, technological developments and the promise of research-focussed space flight have allowed, and will continue to support, a scientific approach to these questions to parallel their investigation by other means.

By 1996, interest in astrobiology was fueled by exciting scientific revelations, including: 1) Images of Europa returned by the Galileo spacecraft indicating the possibility of water under its cracked, icy crust; 2) Detection, in a meteorite from Mars (ALH 84001), of material that could be of biological origin; and 3) Discovery of new planets around extrasolar stars. Later that year, the First Astrobiology Workshop, convened to take an initial step toward the definition of astrobiology, was held at NASA Ames Research Center (ARC) in September.

In March 1997, ARC was designated as the Lead Center for Astrobiology and, in the same year, NASA formalized plans to establish the NASA Astrobiology Institute. In October 1997, a Cooperative Agreement Notice (CAN), a request for research proposals, was issued. More than fifty US research institutions (including universities, free-standing research centers, NASA field centers, and others) responded. The proposed projects were investigator-initiated basic science, but constrained within the wide bounds imposed by the CAN. A requirement for submission was the representation of two or more disciplines in the Team. Hence, from its outset, the interdisciplinary nature of NAI research was established.

In July of 1998, eleven teams were chosen for a five-year funding period of science research and educational outreach programs in astrobiology, and NAI program operations formally began. Also in July, the Astrobiology Roadmap Workshop was held at ARC. This Workshop identified ten science goals, seventeen objectives, and four principles integral to the operation of the astrobiology program. The mission of astrobiology was stated as: "The study of the origins, evolution, distribution, and future of life on Earth and in the Universe." G. Scott Hubbard was appointed as Interim Manager of NAI in August, with attention to implementation of NAI plans. The First NAI General Meeting was held in November 1998 for the NAI Lead Teams to discuss their research projects and ways to develop educational outreach activities.

In May 1999, I accepted the offer to become the Institute's first Director from Dr. Henry McDonald, Center Director of NASA Ames. The office of the Director and his staff – termed NAI Central – was established at ARC. The international reach of astrobiology research was recognized by a formal association with the Centro de Astrobiología in Torreon de Ardoz, Spain.

Excellence in multidisciplinary basic and applied research is the first priority of the Institute; NAI also plays a significant role in training new generations of astrobiologists. A major goal is to respond to the public appeal of astrobiology by building an education and public outreach program to share the excitement of discovery. NAI was established as an interdisciplinary activity and the disciplines traditionally use somewhat different styles of scientific process. The historical sciences – for example, astronomy, geology, and paleontology – are particularly strong on the inductive observation of events that have already happened, while chemical and biological sciences rely to a significant extent on the experimental (and observational) testing of hypotheses. In the inductive phase, data are collected first, then hypotheses are formulated. In the deductive phase, the hypothesis is formulated first, then data are collected to test it. These processes go on sequentially often in a seamless progression, but both processes may proceed at the same time in a complex data set with different hypotheses being formulated and tested concurrently. Astrobiology is a fascinating amalgam of these approaches.

In recognition of the unique nature of astrobiology, and the concurrence of both inductive and deductive approaches within its investigations, I do not expect that the science will progress in accordance with the original proposals or be limited by the Astrobiology Roadmap as written in 1998. This approach allows for the development of new ideas over the period of the five year grants. The future direction of the research is determined by the Members of the NAI Teams and other scientists in the field. Changes in science direction are made by the Principal Investigators, in regular communication with the Director and at annual meetings of the NAI, with the first one held at ARC in November 1998. A meeting of the larger community, the First Astrobiology Science Conference, was held at Ames Research Center in April 2000.

From its origins, NASA has been goal directed and dependent on the successful completion of missions, which, even a few decades ago, would have been considered miraculous. The mission to the Moon was generated, in part, by competition with the former Soviet Union to demonstrate technical and scientific excellence. The goal was evident – it could be seen on most clear nights – and to achieve it required outstanding technical and engineering skills. This can be said for many of the subsequent planetary and outer space missions of NASA and other space agencies. Instruments for scientific discovery are in place, and more will be in the future. Basic scientific research, with a major emphasis on astrobiology, is becoming more and more prominent in NASA and space endeavors. A primary mission of NAI, and astrobiology in general, is to help develop the basic science required for the missions. In time, astrobiology will significantly affect the existing and planned missions and lead to specific missions dedicated to astrobiology questions.

I am pleased to submit to you the Year 2 Annual Science Report (1999-2000). These Team reports detail the scientific progress of Institute-sponsored projects and highlight milestones in the maturation of the NAI as a collaborative global community. This document is only a snapshot of the living work in progress that is the NASA Astrobiology Institute.

AN ANNUAL REPORT OF THE NASA ASTROBIOLOGY INSTITUTE CENTRAL OFFICE JULY 1999-JUNE 2000 AND UPDATE

The Annual Science Report of the NASA Astrobiology Institute appropriately emphasizes the discoveries and research accomplishments of the Institute Members and their Teams. This document also affords an opportunity to record the progress of the Institute in: creating intra- and inter-Institute multidisciplinary communities, contributing to the establishment of astrobiology as an academically valid domain, fulfilling the challenge of innovating scientific collaboration, and addressing the needs of both public and traditional education audiences.

CREATING INTERDISCIPLINARY COMMUNITIES

The Director, through his own efforts and those of his staff at NAI Central, provides the core leadership, continuity, advocacy, communication, and funds support for the Institute's Members and their research activities. NAI was conceived as a 'virtual' collection of remote partners. The NAI's research activities are primarily conducted in the distributed laboratories of the hundreds of Members located at over 80 collaborating institutions and organized through the Principal Investigators (PI's) of the eleven Teams. These PI's, together with the Director and Associate Director, comprise the Executive Council of the NAI.

The overarching shared goal of the NAI and its Members is the conduct of excellent and innovative research in astrobiology, with an emphasis on collaborative work, both within and among the Teams. Focus Groups are a unique example of NAI's collaborative infrastructure. The 'groups' develop around a self-identified topic, generally one that is inherently interdisciplinary, and expand the boundaries of current thinking. These grass-roots proposals are presented to the Executive Council for endorsement. Once approved by the Director, a Focus Group becomes eligible for supplemental funding from NAI Central. Originally implemented as intra-Institute, inter-Team groups, Focus Groups now invite and include many scientists who are not Members of NAI Teams. At present, five Focus Groups are chartered. The NAI Mars (12/99) and NAI Europa (9/00) Focus Groups concentrate on specific planetary exploration objectives. The NAI Mission to Early Earth (12/99), NAI Mixed Microbial Ecogenomics (12/99), and NAI EvoGenomics (4/00) Focus Groups each address innovative and interdisciplinary efforts to synthesize new understanding on topics of current ferment.

Within their Teams and Focus Groups, the Members of the Institute represent a community of broad interests, while the scope of astrobiology guarantees a diversity of expertise. This breadth and diversity, when disparate scientific interpretations arise, can lead to controversy. Within the Institute, the normal scholarly acceptance of controversy is further improved by extraordinary collegial interchange. There is no dilution of individual perspectives, even those at odds, but a continued openness of dialog and cooperative pursuit of mutual goals prevail. This result is all the more notable because it is integral to the Members' appreciation of their communal links, and not the result of any specifically imposed plan or directive.

A further broadening of our collaborative efforts is contributed through our international partnerships. A coincident organization of the NASA Astrobiology Institute is our Associate Member, the Centro de Astrobiología (CAB). Led by Director Juan Perez Mercader, the CAB was initiated in a similar time frame. It is connected to NAI through a Letter of Agreement between NASA and the Instituto Nacional de Técnica Aeroespacial (INTA). This type of negotiated agreement defines Associate Membership. Another form of international partnership, Affiliate Membership, provides for a collaborative connection to the NAI and its extended community without explicit commitments from national space agencies or government institutions. Both Associate and Affiliate Members are invited to participate in NAI workshops, General Meetings, field investigations, Focus Groups, student and senior researcher exchanges, and meetings of

the NAI Executive Council. Recently, we have welcomed two Affiliate Members, the United Kingdom Astrobiology Network (9/00, Dr. D. Cowan, lead) and the Australian Centre for Astrobiology (2/01, Dr. M. Walter, lead).

ESTABLISHING ASTROBIOLOGY

In addition to the Institute's initiation of intra- and inter-Institute partnerships, NASA's institutional sponsorship of NAI demonstrates a unique cross-Enterprise commitment. While NAI's primary and major funding derives from the Office of Space Science, a stable and continuous additional investment has been provided from the Office of Earth Science. Recently, a similar arrangement has been negotiated with the newly re-organized Office of Biological and Physical Research (formerly the Office of Life and Microgravity Sciences and Applications), now an Enterprise level activity. This coalition of funding sponsorship speaks to the strength of the Agency's commitment to the Institute, and the Institute's cross-disciplinary relevance to NASA's research, technology, and mission goals.

Another form of institutional commitment is that provided by the academic and research institutions participating in the NAI. In advocating their selection, our NAI Teams described management commitments ranging from faculty hires and student fellowships to course offerings and the contribution of specialized facilities and equipment. In value, these commitments averaged 20-40% of the final NAI funding provided. At the half-way mark of these five year Cooperative Agreement awards, the anecdotal verdict is that this institutional investment is changing the academic environments of our participating organizations, and contributing to change in the nation's higher education infrastructure. This observation will be quantitatively assessed in the near future.

Finally, it is notable that three independent scholarly publications focusing on astrobiology research are reaching release milestones. Cambridge University Press will publish the *International Journal of Astrobiology* and are soliciting submissions. The first issue of *Astrobiology*, published by MaryAnn Liebert, Inc., has been released. A series of volumes titled *Advances in Astrobiology* has been announced by Springer-Verlag. Many Members of the NAI and its international Associate and Affiliates are prominent on the editorial boards of these publications. Many more will submit their work for dissemination to their peers, and others will act as reviewers. The establishment of a dedicated channel for communicating research results is an important development.

THE CHALLENGE OF COLLABORATION

A key objective for NASA's investment in the NASA Astrobiology Institute is the support of collaborative research involving scientists from multiple disciplines and different institutions. NAI was thus conceived from the beginning as a "virtual institute" in which a variety of communication and collaboration tools, technologies, and processes was to be made available to the geographically dispersed Members. Clearly, the facilitation of new forms of scientific partnership requires active attention to these tools through direct contact and careful tending of the behaviors and preferences associated with productive cooperation. To meet the objective of fostering collaborative multidisciplinary research, we address four priorities: deploying tools (hardware and software), providing training in the use of these tools, creating opportunities for scientific interaction within and among NAI's Teams, and continuously evaluating the operation of collaboration support programs.

A basic information technology infrastructure was deployed for the virtual institute during Year One. This included an ISDN-based videoconferencing system linking the eleven Lead Institutions, NAI Central, and NASA HQ. Electronic "SmartBoards" were installed at these sites, enabling data collaboration via Microsoft's *NetMeeting* application. Team Members not located at a Lead Institution can utilize *NetMeeting* on their desktop computers to collaborate with remote research partners. All Members have

access to *Postdoc*, an ARC-developed document sharing and archiving application accessed through a password-restricted internet site specifically established for NAI. When these collaboration tools were initially deployed, in the spring of 1999, Information Technology (IT) support personnel from ARC visited each Lead Institution to provide training. Training and troubleshooting remains available via telephone and on-line. The combination of videoconference capability, *Netmeeting* data exchange, *Postdoc* document presentation, and teleconferencing supports monthly Executive Council meetings as well as Focus Group collaborations. In addition, two intra-Institute working groups utilize these capabilities, one focused on the Collaborative Research points-of-contact and the other connecting Education and Public Outreach leads.

In addition to these hardware/software tools, collaboration is encouraged in other ways. We have discussed, above, the NAI's Focus Groups. In addition to the monthly Executive Council meetings, three or four in-person meetings of the Council are held at Lead Institution sites each year. The NAI General Meeting is a further opportunity for direct discussion and presentation of findings. This meeting is bi-annual, and it emphasizes the attendance and recognition of students. NAI Central maintains programs supporting inter-Team participation in field investigations and other researcher exchanges. Finally, opportunities for student fellowships are provided. These are discussed in greater detail below.

During Year Two, several significant problems with NAI's collaboration infrastructure were diagnosed and steps have begun to resolve them. These focus on videoconference effectiveness, the *Postdoc* solution, and intra-Team communication. The videoconferencing system has experienced technical problems disruptive to our virtual meetings. Some of these problems stemmed from NAI's reliance on the multicontrol unit (MCU) located at JPL, necessary to support videoconferences involving more than two sites. An MCU was purchased and installed at ARC in July 2000. Technical problems continue to surface, which the NAI IT team is working diligently to resolve. Access to these systems is a recognized concern for Members not located at the Lead Institutions. Distributed desktop tools are needed to facilitate scientific interaction among all Members, and will be implemented and evaluated in the near term.

Postdoc has met with mixed reviews, with negative reactions outnumbering positive ones. A benchmarking study looking at best practices with regard to the sharing and archiving of documents within virtual communities is planned. *Postdoc* may be modified to better meet NAI Member needs or replaced with another system. Strong focus on collaboration across Teams has resulted, for some, in a positive experience of being part of an increasingly cohesive community of researchers. For many Members, however, the lack of focus on collaboration support within their Teams has meant that engagement with geographically distant scientific partners remains difficult. A near-term goal is to improve NAI Central's understanding of the differing work practices of each Team, so that we can address their specific needs and unique challenges. Dynamic adjustments are necessary to enable the Members to interact productively and seamlessly. We recognize the experimental nature of the virtual institute, and we are committed to continuous open dialog.

EDUCATION AND PUBLIC OUTREACH

Second only to providing a collaborative environment for research, training the next generation of researchers and communicating the science of astrobiology are important aspects of NAI's mission. NAI has a vigorous program in Education and Public Outreach (EPO). Aligned with NASA's Strategic and Implementation Plans for education, NAI's EPO program connects scientists with students, teachers, museums, the media, and the general public in exploring astrobiology. Efforts in Education and Public Outreach vary from Team to Team, and they often include vastly different purposes, audiences, and outcomes as well. Expertise varies from Team to Team in different scientific disciplines, as do the EPO programs and projects. However, the individual Teams rely on one another to fill in content that falls out-

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side of their own expertise. NAI Central's Education and Public Outreach program facilitates connections between the Teams, such that the field of astrobiology is accurately and comprehensively represented in the EPO projects and products.

A prime example of this type of collaboration is an educational product for middle school use. During the fall of 1999, the cross-Institute EPO team began work on a set of astrobiology lessons for teachers. The Educator Resource Guide, "LIFE on EARTH...and elsewhere? Astrobiology in Your Classroom" is the result of collaboration among the entire NAI EPO team. Scientists from the Lead Teams of NASA Ames Research Center, Carnegie Institution of Washington, the Jet Propulsion Laboratory, NASA Johnson Space Center, and Pennsylvania State University reviewed content and provided feedback. The science included in the Educator Resource Guide ranges from astronomy to biology, and the five lessons give students and teachers an opportunity to explore astrobiology as a whole. Twenty-two thousand hard copies of the Educator Resource Guide will be produced and distributed at teacher workshops to be held at Lead Team sites as well as at education conferences and meetings around the country. In addition, a pdf version of the Guide is available for download on the NAI website.

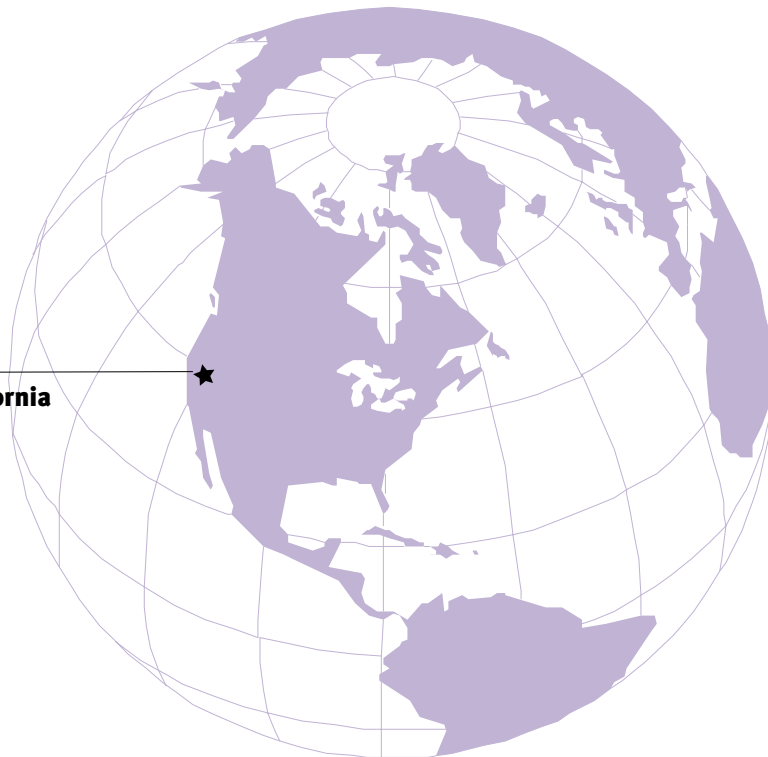
A particular emphasis of our Members is the training of next generation astrobiologists. That training is offered through undergraduate and graduate courses/seminars, certificates and degree programs, and postdoctoral study as well as through educational programs for younger students. NAI Central supports the NAI Postdoctoral Research Associates Program (10-12 two year fellowships), operated by the National Research Council. Six fellowships were awarded in the inaugural year. An additional opportunity for student support is the Director's Scholar program. Travel and Research Scholarships will be available in 2001. Support for the student attendance at NAI's General Meeting is extensive. The large numbers participating in the First General Meeting set a unique tone at the well-attended poster sessions. NAI recognizes the critical role of its students in the life of the Institute, and will continue the tradition of encouraging student participation for the Second General Meeting in 2001.

In addition to work on the Educator Resource Guide, EPO leads and researchers directed and participated in creating, promoting, and producing products and projects that educate in many ways. Between July 1999 and June 2000, the EPO team participated in thirty-five workshops for educators, students, professional scientists, and the general public. In addition, ten astrobiology courses were offered at various universities for graduate, undergraduate, high school, and professional groups. The Lead Teams also participated in seventy-five general public events. Curriculum products and general astrobiology print materials were introduced and distributed at many of the events. Unique programs include twenty-six media projects utilizing television, radio, video, and publications, and three expeditions involving educators and scientists. Particularly worthy of note is the NAI's partnership with NASA's Life Sciences Outreach Program to create and deliver a workshop and materials for K-12 educators coincident with the major professional conference "Bioastronomy '99." As the field of astrobiology takes shape in the scientific community, it is also being formed in the minds of our students, teachers and the general public.

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AMES RESEARCH CENTER

Moffett Field, California



PRINCIPAL
INVESTIGATOR



David
Des Marais

AMES TEAM MEMBERS

Maria Absy,
INPA Manaus

Miriam Absy,
IBAMA Barsilla

Dan Albert,
University of North Carolina

Louis Allamandola,
Ames Research Center

Brad Bebout,
Ames Research Center

Max Berstein,
Ames Research Center

Leonid Brown,
University Of California Irvine

Steven Burton,
USDA, Kansas

Richard Castenholz,
University Of Oregon

Kenneth Cullings,
Ames Research Center

Jeffrey Cuzzi,
Ames Research Center

PROPOSAL EXECUTIVE SUMMARY (1998)

Introduction and Background

The Ames Research Center proposal for NASA Astrobiology Institute membership is based on a broad program of research, training, education, outreach, and service. We are committed to developing the Institute as a revolutionary, multidisciplinary, and virtual organization. As a result of its assigned lead NASA mission in astrobiology, Ames has three distinct roles to play: (1) management and home of the Astrobiology Institute; (2) membership in the Institute, if this proposal is accepted; and (3) non-Institute research and technology development relevant to its astrobiology role. This proposal deals only with item two, namely Institute membership. While the Astrobiology Institute will be managed by the Center, Ames scientists and their associated research facilities will be a part of the Institute only if we are selected through this CAN.

Astrobiology is the primary research activity of the Space Directorate at Ames, and our staff includes world scientific leaders in several astrobiology subdisciplines. In addition, we already have many contacts and collaborations outside of Ames, and in response to this opportunity we are broadening these extramural connections. This proposal has 9 Co-PI's and 57 Co-I's, including 32 Co-I's from 22 other institutions. The broad multidisciplinary nature of the proposed research is indicated by the presence of approximately 40 disciplines. These range from cosmochemistry to astrophysics to atmospheric chemistry to climatology to molecular biology to ecology to microbiology to computer science.

The research plan described here integrates a variety of disciplines along a common theme of origin, evolution, distribution and future of life in the universe. We are able to propose a new program of this scope because of the inherent breadth of our current expertise and research facilities. We are requesting funds under this proposal for new research that is based on activities and infrastructure that are already well grounded at Ames, and we propose a 2:1 ratio of institutional commitments to requested funding. Most of this requested funding goes to extramural colleagues. Also, as the NASA Center of Excellence for Information Technology, Ames will bring modern communication, collaboration, and basic research tools to bear on astrobiology.

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Research

We propose a research program organized around three scientific themes dealing with: (1) the context for life; (2) the origin and early evolution of life; and (3) the future of life as we expand beyond the confines of the Earth. These research themes address all six of the general questions proposed in the CAN (Cooperative Agreement Notice). Within this broad sweep, we are proposing specific, bounded tasks.

Context for Life

We begin with an analysis of the environments conducive to life's origin, addressing the first of the general astrobiology questions from the CAN: How do habitable planets form and evolve? We propose two major initiatives in the cosmochemistry of life. First, we will trace, spectroscopically and chemically, the cosmic evolution of carbon from the interstellar medium to protoplanetary nebulae, planetesimals, and finally onto habitable bodies. Second, we will probe the history of abiotically produced molecules of biological significance. Both initiatives will rely on spectral and chemical studies of realistic, laboratory analogs tightly coupled with quantum chemical calculations followed by astronomical searches. Finally, we turn to the environment in which life develops. Critical to understanding planetary habitability is determination of the extent of the habitable zone around any given star. Requirements are: (1) water must have been delivered to the planet; and (2) climatic conditions must allow surface liquid water to persist. Thus, we focus on the origin and physical state of water. Such study explores the sources of water, the cycling of water and other volatiles between the surface and interior, and the detailed climate of the planet.

Origin and Early Evolution of Life

Do all habitable planets in fact become inhabited? What is the origin and early evolutionary path of life? We focus here on these general questions: How did living systems emerge? and How have the Earth and its biosphere influenced each other over time? In addition, our results will address this question: How can other biospheres be recognized? Our approach to the origin of life is to identify specific segments of this prob-

Hector D' Antoni,
Ames Research Center

Dave Deamer,
University of California Santa Cruz

David Des Marais,
Ames Research Center

Jesse Dillon,
University Of Oregon

Mykell Discipulo,
Ames Research Center

Jason Dworkin,
Ames Research Center

Jack Farmer,
Arizona State University

R. Freedman,
Space Physics Research Institution

Ferran Garcia-Pichel,
Arizona State University

Robert Haberle,
Ames Research Center

Tori Hoehler,
Ames Research Center

Mary Hogan,
University of California Santa Cruz

Miguel-Angel Huerta-Diaz,
University of Ensenada Baja CA

Linda Jahnke,
Ames Research Center

James Kasting,
Pennsylvania State University

Anthony Keefe,
Harvard University

Janos Lanyi,
University Of California Irvine

Carlos Lasta,
INIDEP (Argentina)

Kathleen Londry,
University of Manitoba

Jorge Marcos,
University of Barcelona

Christopher McKay,
Ames Research Center

Betty Meggers,
Smithsonian Institution

Paula Messina,
California State University San Jose

Ellen Metzger,
California State University San Jose

Scott Miller,
Ames Research Center

Michael New,
Ames Research Center

Uli Nuebel,
Montana State University

Simon Payne,
California State University San Jose

David Peterson,
Ames Research Center

lem that are amenable to computational and laboratory investigation. We will create simple biomolecular systems that are capable of performing essential cellular functions, and we will determine conditions under which they can work together in a cellular environment. By building both mathematical and laboratory models of protocellular life, we will test the concept of the most primitive protocells as structures built of evolving components related to those present in contemporary cells, but functioning without genomic control,

Moving from origin of life to early evolution, we will focus on the history of microbial evolution on our own planet. We use a combination of paleohistorical studies with experimental investigation of representative contemporary microbial ecosystems to reconstruct this evolutionary history. An improved understanding of both the long-term evolution of our own biosphere and the biogeophysical cycles that influence the environment will help us assess the prospects for survival of other biospheres and develop a strategy to find them. We seek to understand the origins of early life features that have been preserved, both in the molecular phylogeny of living cells and in the various categories of fossils. The results will include perspectives to assist us in our search for a Martian biosphere and for a model of ancient microbial ecosystems, leading us to predictions of the possible spectroscopic signatures of an inhabited planet.

Future of Life

The final two general astrobiology questions deal with the effect of rapid environmental change on ecosystem properties and the potential for survival and biological evolution beyond the planet of origin. As in the previous themes, we focus our attention on the adaptation and evolution of ecosystems, not individual organisms. In this research, we first use Earth life as a model for life on other worlds, then we consider how terrestrial life could expand beyond its planet of origin, looking specifically at its possible transplantation to Mars. We will study the effects on selected microbial and plant-based systems of varying carbon dioxide concentration, ultraviolet light, and gravity. The results of these studies will be used to create descriptive and predictive ecological models for life under various extraterrestrial conditions.

Training, Education, and Public Outreach

Life in the universe is a subject that interests people of all ages and backgrounds. We propose to use this appeal to develop astrobiology education and public outreach activities, with these goals: (1) enhance science, mathematics, and technology education; and (2) promote the scientific and technological literacy of all Americans. We propose to leverage our extensive existing resources and staff devoted to education and outreach (already mostly funded through other programs), stimulating their focus on new opportunities in astrobiology. Our approach involves: (1) development of source material through a partnership between our scientists and participants in the professional training and development programs; (2) translation of source material into curricula, webpages, public presentations, and other education or public outreach products carried out by professional curriculum developers and communication specialists; and (3) distribution of products to selected target groups (i.e., students, educators, and the public, with special emphasis on reaching under-represented minorities), which will be carried out with our existing infrastructure. Our Co-PI's and Co-I's will each commit 10% of the time spent on this proposed work for educational and outreach activities.

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In K-12 education, we will develop programs for students and teachers to accomplish curriculum development, teacher training, and curriculum dissemination and evaluation. In public education and outreach, we will augment both: (1) existing mechanisms developed by the Ames External Affairs Office to provide a resource for life-long learning; and (2) an informative forum for public participation in the scientific discovery process in astrobiology. We have an outstanding history of professional training related to astrobiology: 125 two-year National Research Council post-docs in the past decade, 28 of whom are Co-I's in this proposal. We will enhance an existing Stanford University course in Astrobiology and Space Exploration, making it available to a much broader audience through the Web and other means of dissemination. Also, we will develop and teach a summer course for graduate students in astrobiology, designed to develop future astrobiologists with greater skill in multidisciplinary research.

Management, Institutional Commitment, and Budget

Ames is submitting a single integrated proposal that involves 66 investigators, with 32 of them from other institutions. Although we have been involved for years in astrobiology-related research, never before have our separate disciplines and organizational structures been brought together so directly in pursuit of common objectives. The PI, David Des Marais, has overall authority and responsibility, supported by Donald DeVincenzi (Co-PI) who is responsible for day-to-day management. Individual research and education/outreach tasks are the responsibility of the other seven Co-PI's. We describe plans for multidisciplinary and multi-institutional communication and integration, based in part on innovative uses of modern technology. The Space Directorate at Ames further commits to the institutional support and oversight of this broadly-based effort.

As NASA's lead center, Ames has a large stake in astrobiology. We have hired 7 new civil service astrobiologists in this past year (FY97), with 5 of them appearing as Co-I's on this proposal. We commit to hire at least 5 more civil servant astrobiologists in FY98 to support this proposed work. Except for students and post-docs, most of the workforce to carry out the research and education/outreach work proposed here is offered at no cost under institutional cost-sharing. Facilities and other infrastructure also do not appear as charges against this funding. Our total cost-sharing is \$10.5M in the first year and \$36M for 5 years. In comparison, the requested funds under this proposal are \$2.4M in the first year and \$14.4M over 5 years, primarily for the support of extramural collaborators. We also bring to the Institute, at little or no direct cost, a number of state-of-the-art computational and information technology tools developed at Ames to enhance and facilitate research and help bind together researchers at dispersed geographical locations.

P Pilewskie,
Ames Research Center

Andrew Pohorille,
Ames Research Center

Chris Potter,
Ames Research Center

Lee Prufert-Bebout,
Ames Research Center

M. Rabbette,
Ames Research Center

Christopher Raleigh,
Ames Research Center

R. Pamela Reid,
University of Miami

Bruno Risatti,
Illinois State Geological Survey

Daniel Rogers,
University Of Connecticut

Nina Rosenberg,
Lawrence Livermore National Laboratory

Scott Sanford,
Ames Research Center

N. Sleep,
Stanford University

Dimitris Stassinopoulos,
Ames Research Center

Roger Summons,
Australian Geological Survey Organization

Jack Szostak,
Harvard University

Margaret Tolbert,
University Of Colorado Boulder

Brian Toon,
University Of Colorado Boulder

Kendra Turk,
University of California Santa Cruz

Pieter Visscher,
University Of Connecticut

David Ward,
Montana State University

Michael Wilson,
Ames Research Center

Richard Young,
Ames Research Center

Kevin Zahnle,
Ames Research Center

Richard Zare,
Stanford University

M. Zolensky,
Johnson Space Center

Roadmap Objectives

#1

Sources of Organics
on Earth

#2

Origin of Life's Cellular
Components

#11

Origin of Habitable
Planets

#13

Extrasolar Biomarkers

Project

Chemical Building Blocks

Senior Project Investigator(s):
Louis Allamandola

ACCOMPLISHMENTS

We are making significant progress in our studies on the photochemistry of realistic interstellar and cometary ice analogs. Ultraviolet irradiation of these ices at temperatures close to absolute zero (the temperature in deep space) produces a mixture of organic molecules that are far more complex than the simple parent species. We believe this mixture (a refractory organic residue) is similar in nature to the complex organics that potentially rain down on habitable planets. These complex organics may be carried in interplanetary dust particles (IDPs), comet dust particles, and perhaps even in some meteorites. The key question is, "Could these have played a role in the origin of life?" Until recently, this seemed highly speculative and, perhaps, unlikely. This idea is now being reconsidered, and our results are an important part of this endeavor.

Our ice studies proceed along two fronts, addressing ices containing polycyclic aromatic hydrocarbons (PAHs) and ices without them. PAHs are widespread throughout the interstellar medium. We are the first to study their ice photochemistry under interstellar and early Solar System conditions. In non-PAH containing ices, we find that compounds are produced, which self-organize into vesicles (primitive protocells) when the residue is exposed to liquid water, in a fashion similar to that when an IDP falls down into water on a habitable planet. Further, other compounds produced luminescence by capturing ultraviolet light and internally converting that energy into other states. That is, we observed compounds that have the ability to harvest energy from the environment. Both vesicle formation and energy harvesting are considered critical in the origin of life, and we have shown that this chemistry takes place under the harshest conditions imaginable in deep space. The implication is that this level of chemical complexity is widespread throughout the cosmos. We do not yet know the identity of these compounds and have just started this aspect of the investigation, looking specifically for biogenic compounds. In our study of PAH photochemistry in water ice, we have observed production of biogenic compounds used in living systems today. All of these results have very important implications for the origin of life.

HIGHLIGHTS

- We have shown that, under the extremely cold, harsh conditions in deep interstellar space, vesicle-forming compounds are readily made. When these are exposed to liquid water, they self-organize into vesicles with primitive protocell properties.
- We have shown that, under the extremely cold, harsh conditions in deep interstellar space, compounds are produced that can both harvest light energy from the surroundings and concentrate that energy in the primitive protocells.
- When polycyclic aromatic hydrocarbons (PAHs), compounds widespread throughout

the interstellar medium, are included in our interstellar/precometary ice simulations, we have observed production of several biogenic compounds used in living systems today.

Habitable Planets

Project

Senior Project Investigator(s):
Richard .E. Young

ACCOMPLISHMENTS

Our first geochemical modeling study of the aqueous alteration of CM parent bodies was completed. Some of these results were presented at the April 2000 *Astrobiology Science Conference* at Ames. A manuscript reporting on the study was just completed and is being submitted to *Meteoritics & Planetary Science (MAPS)* (Rosenberg, Bourcier, and Browning). Results support a scenario in which both low-temperature aqueous alteration of an anhydrous CM parent body plus melt water from H₂O and CO₂ ices produce the altered assemblage observed in CM carbonaceous chondrites.

A model has been developed in which atmospheric carbon dioxide (and therefore climate) is controlled by continental and seafloor weathering. This model predicts that Earth would have been cold in the Archean if there were no other greenhouse gases. A paper reporting this work by Sleep and Zahnle has been submitted to *Journal of Geophysical Research*. A sample from a 3.85 billion year old Banded Iron Formation has been analyzed in a search for meteoritic material. Little was found, which can be explained in part by rapid sedimentation and in part by sampling biases inherent in power law distributions. A paper describing this work was submitted to *Journal of Geophysical Research* by Anbar, Arnold, Mojzsis, and Zahnle.

Laboratory and theoretical studies were accomplished concerning how carbon dioxide ice clouds affect the greenhouse effect, and hence the outer boundary of the habitable zone. In work done at Pennsylvania State University, CO₂ clouds were shown to be strongly warming, but these clouds can cool the surface if they are low and/or optically thick. A paper summarizing this work was submitted by Mischna, Kasting, Pavlov, and Freedman to the journal *Icarus*. Laboratory work investigating cloud condensation is being conducted at the University of Colorado. This work provides critical information on cloud condensation microphysics, needed to model carbon dioxide clouds in early atmospheres of Mars and possibly Earth, as well as in the current atmosphere of Mars. A paper on this work has been submitted to *Geophysical Research Letters* by Glandorf, Colaprete, Tolbert, and Toon. A study of the radiative fluxes in the Earth's atmosphere over the Pacific Ocean has been initiated at Ames. This study was stimulated because satellite observations indicate the signature of the runaway greenhouse effect in localized regions, along with an unidentified mechanism (perhaps clouds) that limits sea surface temperature. Initial radiative transfer calculations show that with sufficient and rea-

Roadmap Objectives

#11

Origin of Habitable Planets

#12

Effects of Climate & Geology on Habitability

sonable amounts of water vapor distributed appropriately, a maximum is reached in the emitted upward IR flux at the top of the atmosphere, consistent with the satellite observations. An abstract and poster on this work appeared at the *First Astrobiology Science Conference* (April 2000) by Rabbette, McKay, Pilewskie, and Young.

In the next year, we plan to take advantage of MOR.ICE, our new modeling code. We will use it to explore water redistribution throughout a planetary body as it heats, influenced by several conditions: (1) the assumed thermal source responsible for supplying the heat; (2) the size of the parent body; (3) rock properties such as porosity and permeability; and (4) the initial quantity and distribution of ice. We will develop a more detailed description of the thermal conditions at the surface of the earliest Hadean Earth, with the focus on global conditions likely to have influenced the origin and earliest evolution of life. A parameterized carbon dioxide cloud microphysics model and a detailed radiative transfer model will be incorporated into an existing 3-dimensional circulation model of the Martian atmosphere, in order to assess fully the importance of CO₂ ice clouds on past and current Martian climate. This is in effect a study of the outer boundary of the habitable zone in our solar system. We will continue investigating the radiative balance over the Earth's oceans, and the implications the observed balance has for understanding the runaway greenhouse effect and processes that might place limits on it.

HIGHLIGHTS

- A model has been developed for the early Earth in which atmospheric carbon dioxide (and therefore climate) is controlled by continental and sea floor weathering. This model predicts that Earth would have been cold in the Archean if there were no other greenhouse gases. To understand the faint young sun problem on early Earth probably requires considering additional greenhouse gases besides carbon dioxide, together with identifying a way to keep these additional gases from being photochemically destroyed on relatively short time scales.

Carbon dioxide ice clouds have been suggested as a means to enhance the greenhouse effect and keep early Mars warm. Whether carbon dioxide ice clouds heat or cool a planetary surface depends on the opacity and location of the clouds. Carbon dioxide ice clouds can enhance the greenhouse effect and thereby warm a planet's surface, but the clouds can cool the surface if they are low and/or optically thick. It may be difficult to form high and/or thick carbon dioxide cloud layers because laboratory experiments show that the cloud particles tend to be large and fall out of the atmosphere relatively quickly.

Cross Team Collaborations

We have been actively collaborating with Jim Kasting (Pennsylvania State University) as well as Brian Toon and Maggie Tolbert (University of Colorado) on studies of the microphysics and radiative transfer properties of CO₂ ice clouds. These collaborations will result in the incorporation of their microphysics and radiative transfer models into the Mars circulation model developed at Ames. We can then assess whether CO₂ ice clouds might have been a significant factor in determining the outer boundary of the habitable zone in our solar system.

Early Metabolic Pathways

Project

Senior Project Investigator(s):
Andrew Pohorille

ACCOMPLISHMENTS

The main, long-term goals of this work have been to: (1) develop protein enzymes representative of those that existed on the early Earth; (2) couple their catalytic activity in model protocells to external sources of energy and nutrients; and (3) determine conditions under which such protocellular systems can evolve using theoretical modeling. The selection of four new adenosine triphosphate (ATP)-binding proteins from six trillion random polypeptides has been completed. The sequences of these proteins are not related to any known biological proteins. As such, they are the first truly novel proteins of non-genomic origin. Deletion studies revealed that the minimal binding unit is less than 50 amino acids long. Thus, it is the smallest known ATP-binding protein. The results demonstrate that the method of in vitro selection of proteins is successful and general. Therefore, it can be used to select proteins with different activities. The method also provides a unique tool to study early evolution of proteins.

A simple bioenergetics system was built, consisting of bacteriorhodopsin and ATP synthetase incorporated into phospholipid liposomes. The ability of this system to synthesize ATP in response to light was demonstrated and optimized. Further, ATP generation was coupled to synthesis of acetyl coenzyme A. This represents a simple system in which environmental energy drives an essential metabolic reaction.

It was demonstrated that mixed short chain monocarboxylic acids and alcohols can form stable membranous vesicles that retain macromolecules such as DNA. Thus, they represent plausible models for early membranes. It was further shown that simple membranes can be sufficiently permeable to nutrients to support template-directed synthesis of RNA. This result demonstrates that once genetic material has been entrapped within the boundaries of a protocell, replication is possible using external sources of nutrients and energy.

A simple model of early evolution of protocells in the absence of genome was investigated in detail. A community of protocells containing proteins only (specifically, proteins capable of both forming and breaking peptide bonds) was studied. Such a protocell community was observed to increase its catalytic potential under a wide range of assumptions about the distribution of catalytic activities in random proteins. This suggests that non-genomic evolution may be a robust phenomenon.

Plans for the next year include the following: (1) determination of the three-dimensional structure of the isolated, novel protein; (2) initialization of new selection processes; (3) optimization of coupling between bioenergetic systems and metabolic reactions, as well as their encapsulation in a model protocellular environment; and (4) development of advanced theoretical models of non-genomic evolution of protocells, that incorporate

Roadmap Objectives

#2

Origin of Life's Cellular Components

#3

Models for Life

several essential protocellular functions.

HIGHLIGHTS

- ‘First-ever’ functional proteins were selected *in vitro* from a very large collection of random proteins. They may represent the first examples of proteins that may have existed on the early Earth. *In vitro* selection of proteins also has an immense biotechnological potential by providing tools to develop novel proteins with specificities not available in cells.
- It was demonstrated how simple metabolic reactions and synthesis of biopolymers can be coupled to external sources of energy and nutrients. This coupling was a key step in the evolution from inanimate to animate matter.
- A theoretical model was developed, which demonstrates how a simple protocell can evolve in the absence of a genome. It is asserted that the diversity of catalytic activities rather than self-replication is necessary for the early evolution of protocellular systems.

Cross Team Collaborations

In collaboration with researchers associated with the Scripps Institution, a proposal for the Origin of Metabolic and Replication Systems Focus Group has been developed. This focus group will include scientist teams from Scripps, Ames, University of Colorado, and the Los Alamos/Santa Fe Institute. As the first step, a joint proposal between experimentalists and theorists at Scripps and Ames has been developed and submitted to NAI Central. This proposal is devoted to determining one of the critical parameters of protobiological evolution, namely the distribution of catalytic activities of random polymers.

Roadmap Objectives

#5
Linking Planetary &
Biological Evolution

#6
Microbial Ecology

#7
Extremes of Life

#13
Extrasolar Biomarkers

Project

Early Microbial Ecosystems: Modern Analogs

Senior Project Investigator(s):
David Des Marais, Brad Bebout, Pieter
Visscher, Richard Castenholz, David Ward

ACCOMPLISHMENTS

We examine the roles played by microbial ecological processes, both in creating biological markers (e.g., gases, chemical compounds, and morphologic features in sediments) and in influencing the adaptation and evolution of microorganisms.

In microbial mats at Guerrero Negro, Mexico, the budget of oxygen and carbon varied seasonally, probably due to changes in the balance between gross and net mat productivity. Significant methane production occurred in the surface layers of subtidal mats, but production was very low in intertidal mats. Substantial hydrogen production occurred in mat surface layers, and intertidal mats generated larger fluxes than the subtidal mats. In mat porewaters, significant concentrations of the following acids were

observed: lactate, acetate, formate, and propionate. These acids did not escape into the overlying water column under oxic conditions, but they did escape under anoxic conditions. The flux of dimethylsulfide from subtidal mats was significant in June, but it was very small in October. A variety of low molecular weight thiol biomarkers was also found. In our studies, almost half of the cyanobacteria synthesize 2-methyl hopanoid, which appears to be uniquely diagnostic of cyanobacteria. This was recently identified by some of us in 2.7 billion-year-old rocks. *Phormidium* cyanobacteria from Yellowstone exhibit at least four clades (taxonomic groups) of distinct genetic types with considerable genetic distance between them. This indicates a split of >>100 million years among them. Within this assemblage are three distinct clades that correspond to different lipid biomarkers, including 2-methylhopanoid. Studies of 16S rRNA in green nonsulfur bacteria in Guerrero Negro mats revealed a previously undocumented cluster of organisms that is deeply divergent from previously-described populations. Ultraviolet light substantially affects the net production of oxygen by intertidal mats at Guerrero Negro.

A microbial mat greenhouse facility was established for post-field trip ongoing research and for integration with information systems technology at Ames. Cyanobacterial culture collections are being maintained and cross-referenced to increase their accessibility to the astrobiology community. We will explore the impact of environmental parameters such as season, temperature, sunlight, and oxygen availability upon the structure and function of subtidal and intertidal mats, with an emphasis on their production of biomarker compounds, including gases. We will conduct parallel genetic studies of photosynthetic populations to establish relationships between population richness, biomarkers, and ecosystem function.

HIGHLIGHTS

- **Reduced Gas Emissions**

Cyanobacterial mats have been demonstrated to produce volatile hydrocarbon and sulfur gases that might have constituted important contributions to Earth's early atmosphere. Accordingly, microbial communities will serve as useful objects of study to understand the nature of biomarker compounds that might be detected spectroscopically in remote atmospheres. Specifically, methane and dimethylsulfide could be considered as promising candidate biomarker compounds.

- **Cyanobacterial Biomarkers**

Cyanobacteria are a supremely important group evolutionarily, because they "invented" oxygenic photosynthesis. Their evolution on early Earth is important, both for the history of our atmosphere and for the levels of global productivity of our biosphere. Our work with cyanobacterial biomarker lipids is creating a set of molecular indicators for charting the rise and diversification of microbial ecosystems driven by oxygenic photosynthesis. Ongoing work in our group is testing the specificity of previously-identified cyanobacterial biomarkers. We are also searching for biomarkers not yet recognized as valuable indicators of cyanobacteria and other photosynthetic populations. Parallel genetic studies of cyanobacteria are being conducted to determine the phylogeny of cyanobacterial lipid biomarkers.

- “Archean Gardens” Mat Greenhouse

Field work continues to be the cornerstone of our research. However, the “Archean Gardens” greenhouse at Ames allows us to extend our observations to include manipulations of mats under conditions not attainable in the field environment. This will become increasingly important as we try to understand how microbial ecosystems functioned under environmental conditions on the early Earth or, perhaps, beyond Earth. The presence of mat ecosystems at Ames, where information and microsensor science and technology are being advanced, creates valuable opportunities to develop new concepts and methods for microbial ecological research. The “Archean Gardens” established at Ames has already been used successfully for studies of mats returned from our Guerrero Negro field site.

Field Expeditions

In this NAI Year 2, two major field trips were conducted to Guerrero Negro, Baja California Sur, Mexico.

- Attendees at the October 7 to 20, 1999 trip were: D. Albert (University of North Carolina); B. Bebout, D. Des Marais, T. Hoehler, S. Miller, C. Raleigh (NASA Ames Research Center); J. Dillon (University of Oregon); M. Huerta-Diaz, C. Nava Lopez (University of Ensenada, Baja CA); J. Risatti (Illinois Geographical Survey); and D. Rogers (University of Connecticut).
- Attendees at the May 15 to 29, 2000 trip were: B. Bebout, B. Blumberg, D. Des Marais, T. Hoehler, S. Miller, C. Raleigh (NASA Ames Research Center); J. Dillon (University of Oregon); F. Garcia-Pichel, J. Farmer, M. Farmer (Arizona State University); M. Hogan, K. Turk (University of California-Santa Cruz); M. Huerta-Diaz, A. Siequeiros (University of Ensenada, Baja CA); and P. Visscher (University of Connecticut).

Cross Team Collaborations

Arizona State University and NAI Central

A field trip to Guerrero Negro, Baja California Sur, Mexico in May 2000 was the beginning of an interdisciplinary study of the hypersaline microbial mats at Guerrero Negro. This study carries implications, both for our understanding of early microbial evolution and for the interpretation of biomarker gases and sedimentary organic matter.

MBL, Arizona State University, and Colorado Teams

This ecogenomics focus group, just beginning, will address the structure and function of microbial communities from these three perspectives: biogeochemistry, gene expression, and population dynamics at the ecosystem level.

Harvard Team

The Chuar Project is an interdisciplinary study of the sedimentology, paleontology, and geochemistry of the ca. 850 million-year-old Chuar Group in Grand Canyon, AZ. A related paper is in press in the journal *Geology*. This research documented the events associated both with breakup of the Neoproterozoic supercontinent Rodinia and with the onset of a major period of glaciation.

Rapid Rates of Change

Project

Senior Project Investigator(s):
Hector D'Antoni

ACCOMPLISHMENTS

In this period, a remote sensing database has been compiled to study the tropical rain-forest. This database is composed of Landsat TM (Landsat Thematic Mappers) data at a space resolution of 30 meters, with 7 bands from visible to infrared radiation reflection. Additionally, a radar database was compiled (NASDA, Japan: National Space Development Agency of Japan), and some high resolution data are currently being obtained for specific regions. Due to limited funding, we have continued establishing collaborative work with colleagues working in South America. We have concentrated on Ecuador, where a major grant was awarded to our Co-Investigator, Dr. Jorge Marcos (University of Barcelona, Spain).

Our analyses of a 15-year AVHRR (Advanced Very High Resolution Radiometer) database find NDVI (Normalized Difference Vegetative Index) variations for each of the involved ecosystems in El Niño and non-El Niño years. These studies prepare for calibration of modern pollen spectra in terms of the spectral signature of extant vegetation. Data analysis has been performed for Central Argentina (heavily affected by El Niño) and West and Southern Argentina (non-El Niño region, control). Results support our central hypothesis that El Niño effects can be discerned from changes in the spectral signature of vegetation. A paper authored by D'Antoni, Mezger, and Payne is currently in preparation.

In our cooperation with scientists from Brazil, we have jointly established a remote sensing database. We are using these data collection systems: AVHRR, Landsat MSS (Landsat Multispectral Scanner), and NASDA Radar. Twenty percent of the data under calibration now are from Brazil.

HIGHLIGHTS

- Results support our central hypothesis that El Niño effects in South America can be discerned from changes in the spectral signature of vegetation. This enables our calibration of modern pollen dispersal data, in terms of vegetation remote sensing data. This is a second step toward our objective of retrogressive studies of biogeochemical cycles.

Field Expeditions

Following the site visits in Argentina and Brazil by David Peterson and Hector D'Antoni (April 1999), D'Antoni visited Patagonia in January 2000. This resulted in findings that opened another line of research, which has been recently funded. It also improved interaction with Argentine colleagues at University of Buenos Aires (Dr. J. A. Perez-Gollan, Dr. Nora Madanes, and Dr. Patricia Kandus), and in Patagonia (Dr. Eduardo Musacchio,

Roadmap Objectives

#14

Ecosystem Response to Rapid Environmental Change

and Dr. Nilda Weiler). Field measurements of reflectance were taken in Patagonia to use in Landsat image calibration. Other collaborations are being established as a result of the above-mentioned fieldwork sessions. These will contribute research at no cost to our module: Dr. J.A. Perez-Gollan (University of Buenos Aires), Lic. Carlos Ceruti (Museo Antonio Serrano, Santa Fe).

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ARIZONA STATE UNIVERSITY



Tempe, Arizona

**PRINCIPAL
INVESTIGATOR**



**Jack
Farmer**

ASU TEAM MEMBERS

James Allen,
Arizona State University

Alice Baldrige,
Arizona State University

Josh Bandfield,
Arizona State University

Carl Bauer,
Indiana University

Brad Bebout,
Ames Research Center

Gretchen Benedix,
Arizona State University

Robert Blankenship,
Arizona State University

Philip Christensen,
Arizona State University

George Cooper,
Ames Research Center

Marina Cosarinsky,
Arizona State University

Don Crampton,
Arizona State University

PROPOSAL EXECUTIVE SUMMARY (1998)

Arizona State University seeks to enhance the newly emerging field of astrobiology through membership in NASA's Astrobiology Institute. The research program we are proposing is broad in scope and addresses many of the fundamental questions posed in the CAN (Cooperative Agreement Notice). ASU's proposal is motivated by years of involvement with NASA's planetary programs and missions, unique strengths in evolutionary life sciences, strategic collaborative partnerships, and a desire to stay on the cutting edge of planetary science.

The first of five proposed research modules seeks to delineate the ongoing controversy over the relative contributions of endogenous and exogenous processes in the origin of basic precursor molecules for life. The first research task of Module 1 will be led by John Cronin and Laurie Leshin and will focus on the organic chemistry of carbonaceous chondrites and interplanetary dust particles (IDP's). These are the most important sources of organic matter delivered to the Earth from the interplanetary environment. Materials for study will mostly be drawn from ASU's Meteorite Center, which maintains the largest University-based collection of meteorites in the world. The work will be carried out in close collaboration with scientists from the Exobiology Branch of NASA Ames. The proposed work involves a close synergy between several disciplines and the application of new methods of analysis.

The discovery ~twenty years ago of chemotrophic communities living around deep sea vents led to a new paradigm for the endogenous production of prebiotic organic materials on Earth and the origin of life. It has now been shown theoretically that hydrothermal environments provide favorable conditions for the synthesis of the organic compounds required for life. This has strengthened the case that life could have originated at high temperatures, a radical departure from the traditional view. The second research task of Module 1 will examine the concept of prebiotic synthesis at high temperatures using a new experimental "black smoker system" designed by Co-Investigator John Holloway. This experimental system will be used to test thermodynamic predictions regarding the catalytic synthesis of simple organic compounds from common inorganic molecules, either found in seawater or released by hydrothermal alteration of sea floor basalts. During the course of the proposed study, conditions will be varied to

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model hydrothermal sites on Mars and Europa, thus helping to constrain the possibilities for life on those bodies.

Research Module 2 picks up the theme of early biosphere evolution by focusing on the origin and evolution of photosynthetic systems. This effort will be coordinated by Robert Blankenship (Director of ASU's Center for the Study of Early Events in Photosynthesis). The origin of oxygenic photosynthesis marks one of the most important biochemical events in the history of the biosphere. With acquisition of the ability to oxidize water, organisms began to release molecular oxygen into the oceans and atmosphere. This eventually changed the redox balance of the Earth and provided a sufficiently-oxygenated atmosphere to sustain complex multicellular forms of life. Emphases in the proposed research are: 1) studies of photosystems in what are generally presumed to be more primitive bacterial groups with simpler systems of anoxygenic photosynthesis; and 2) the discovery of new classes of primitive photosynthetic organisms. The evolutionary relationships between photosynthetic groups will be studied by sequencing genes from new groups and comparing them to existing sequence data for known groups. The horizontal transfer of genetic information has been recently realized to be a fundamental factor in the organization of the microbial genome. Could the horizontal transfer of the genes for photosynthetic reaction centers provide an explanation for observed phylogenetic patterns? This possibility will be studied in collaboration with Peter Gogarten (University of Connecticut) and others. We also propose to use artificial photosynthetic membranes to model the simplest known forms of photosynthesis. This approach is based on photo-induced electron transfers within protein complexes that generate a redox potential across membranes. Such processes are central to the bioenergetics of living cells and will aid our understanding of how the first photosynthetic systems operated. These studies also have direct applications in evaluating the potential for photosynthetic systems to develop within other planetary environments, such as Mars and Europa, and may be of interest in designing photosynthetic systems for use during human colonization of space. In a much broader context, artificial membrane studies could someday impact our interpretations of oxygenic signatures detected in the atmospheres of extrasolar planets elsewhere in our galaxy.

In Module 3 the proposed research will move away from the historical record of evolu-

John Cronin,

Arizona State University

David Des Marais,

Ames Research Center

Thomas Dowling,

Arizona State University

Eileen Dunn,

Arizona State University

James Elser,

Arizona State University

William Fagan,

Arizona State University

Sarah Fagents,

Arizona State University

Jack Farmer,

Arizona State University

James Farquhar,

University of California San Diego

Patricio Figueredo,

Arizona State University

Tom Folz,

Arizona State University

Wayne Frasch,

Arizona State University

Ferran Garcia-Pichel,
Arizona State University

Darcy Gentleman,
Arizona State University

Peter Gogarten,
University Of Connecticut

Ronald Greeley,
Arizona State University

Victoria Hamilton,
Arizona State University

John Holloway,
Arizona State University

Paul Knauth,
Arizona State University

David Kring,
University Of Arizona

Vanessa Lancaster,
Arizona State University

Laurie Leshin,
Arizona State University

Jay Melosh,
University of Arizona

Jeffrey Moersch,
University of Tennessee

Thomas Moore,
Arizona State University

John Moreau,
Arizona State University

David Nelson,
Arizona State University

Peggy O'Day,
Arizona State University

Meredith Payne,
Arizona State University

Beverly Pierson,
University of Puget Sound

tion contained in the genetic sequences of living organisms to the more direct record of evolution provided by fossils. The main focus of this research will be to combine studies of fossilization processes in modern microbial systems with cutting-edge methods of electron microscopy. Studies of modern fossilization processes will be led by Jack Farmer, who will compare three types of environments (thermal springs, marginal marine, and lacustrine). The work will interface with a larger interdisciplinary study being proposed by a microbial ecosystems group led by David Des Marais (NASA Ames Research Center) and a second interdisciplinary ecosystems group at ASU lead by James Eiser (Module 4). This research includes a variety of field- and lab-based components and involves microbial ecologists, biogeochemists, molecular biologists and paleontologists.

The second task of Module 3 will be coordinated by Thomas Sharp (ASU), who will study the microstructure of siliceous microfossils. Most of the well-preserved fossil microbios in the Precambrian are hosted in cherts (silica-rich sediments). The goal is to apply state of the art methods of electron microscopy and chemical microanalysis to evaluate the nano-scale structural features of Precambrian microfossils and reconstruct the important events in fossilization. This will enable the development of methods and criteria for evaluating the biogenicity of nano-scale features in ancient sediments. In the third task of Module 3, Paul Knauth will lead an effort to use data from the geological record to reconstruct the salinity of the Archean oceans. Prior to the origin of stable continental platforms, it would have been impossible to sequester salt from the oceans on the continents. Preliminary work suggests that if all the salt stored on the continents as evaporite deposits, or as subsurface brines, were put back into solution in the oceans, the salinity would rise by 1.3 to possibly 2.0 times present seawater values. This finding has important implications for the origin of life and early evolution of the biosphere. It also bears on the question of life on Europa or other bodies in the solar system where saline oceans may exist.

In Module 4, James Eiser will lead an interdisciplinary team to study the evolution of diversity in mat-snail-fish ecosystems living in arid spring environments. The site for study is Cuatro Ciénegas in central Mexico. This area harbors a large number of extreme aquatic environments (including thermal, hypersaline and alkaline), which have been in existence for more than 40,000 years. At the top of the food chain are species of cichlid fish that have diversified into a large number of species through trophic and reproductive specialization. The first-level consumers in these systems include many species of snails that graze on stromatolite-forming microbial mats. Mat systems are dominated by cyanobacteria and diatoms, which appear to induce the precipitation of carbonates by their photosynthesis. All three groups have an extensive fossil record preserved in the sediments of the basin, which provides access to their long-term evolutionary history. An interdisciplinary study of the ecology, molecular systematics, aquatic geochemistry, and paleontology of these systems is proposed to determine the mechanisms of diversification within a well-defined context of environmental changes, including those due to biological factors (e.g., competition for resources).

In Module 5, the emphasis is on the search for past or present life elsewhere in the solar system. The proposed targets for study are Mars and Europa. The Mars effort will be led by Phil Christensen, Jack Farmer, and Ron Greeley. Christensen is Principal

Year 2

Investigator (PI) for the Thermal Emission Spectrometer (TES) experiment, which is presently orbiting Mars. He is also PI for the Thermal Emission Imaging System (THEMIS, an orbital instrument that will be launched to Mars in 2003) and the mini-TES experiment (a landed instrument for the 2001 opportunity). All of these instruments will provide information about the mineralogy of the martian surface, the most important step in initiating a program to explore for a martian fossil record. Combining skills in spectroscopy and mineral mapping (Christensen) with those in photogeologic interpretation (Greeley) and microbial paleontology (Farmer), this group will work with data from upcoming Mars missions. These efforts are combined to understand more about distribution of environments that could have once sustained life and to develop site selection criteria for optimizing future surface robotic and sample return missions.

In task 2 of Module 5, Ron Greeley will lead an effort to evaluate the potential for habitable environments on Europa. This will involve analysis of data from Galileo and the Europa Extended Mission, along with theoretical and empirical modeling (using Earth analogs) to understand the most energetically favorable environments and likely metabolic strategies for a putative Europa life form(s). Collaborators include Chris McKay (NASA Ames) and Jack Farmer, who will provide expertise in exobiology. The proposed work will lay a foundation for site recommendations to support future orbital and robotic surface missions to explore for evidence of extant life in subsurface habitats or cryopreserved fossil materials in surface ice deposits.

Our plan for training includes yearly stipends to support: (1) research assistantships at all levels; (2) a core course and seminar series in astrobiology that will be taught by a distributed faculty drawn from institute partners; and (3) a virtual classroom and lab that will provide an opportunity for remote learning for credit. There will also be a degree emphasis in astrobiology stressing interdisciplinary studies. Institutional commitments include two new faculty hires and a staff position in cosmochemistry and exobiology, beginning in the Fall of 1998, plus two additional faculty hires in microbial ecology and evolution during the next five years. In addition, ASU will establish a Center for Astrobiology that will: (1) administer and manage grant funds; (2) supervise curriculum development; (3) coordinate use of NGI to support real-time collaborative research and training; and (4) interface with the Astrobiology Institute to help sponsor on-line workshops, seminars, and other means of sharing knowledge. The ASU Astrobiology Center will enhance well-established planetary-based public outreach activities in Arizona, including those related to the Mars Global Surveyor and Galileo missions, as well as those of the Jason Project, Biosphere 2, and ASU sponsored annual outreach events.

The PI will have responsibility for overall project direction, but an advisory board consisting of Module leaders will make project management decisions. Also, the PI will establish a monthly meeting schedule to promote ongoing dialog among collaborators. Group interactions will also be encouraged through an astrobiology seminar series and by electronic dissemination of quarterly highlights by Module leaders, who will report progress in their respective project elements. Coordination of integrated research efforts will be overseen by the PI with help of a full-time administrator, who will help carry out the routine activities of the Astrobiology Center and interface on a regular basis with the NASA Astrobiology Institute.

Sandra Pizzarello,
Arizona State University

Gary Plumley,
University Of Alaska

Jason Raymond,
Arizona State University

Steve Ruff,
Arizona State University

Steve Scotnicki,
Arizona State University

Thomas Sharp,
Arizona State University

Carol Tang,
Arizona State University

Mark Thiemens,
University of California San Diego

Cindy VanDover,
College of William and Mary

Hebe Vanegas,
Arizona State University

Kenneth Voglesonger,
Arizona State University

Terri Williams,
Arizona State University

Roadmap Objectives

- #1 Sources of Organics on Earth
- #2 Origin of Life's Cellular Components
- #3 Models for life
- #4 Genomic Clues to Evolution
- #5 Linking Planetary & Biological Evolution
- #6 Microbial Ecology
- #7 Extremes of Life
- #8 Past and Present Life on Mars
- #9 Life's Precursors & Habitats in the Outer Solar System
- #12 Effects of Climate & Geology on Habitability

Project

Exploring the Living Universe: Origin, Evolution, and Distribution of Life in the Solar System

Senior Project Investigator(s):
Jack Farmer

ACCOMPLISHMENTS

ASU's commitment to establish a new, cutting edge ion probe facility in the Geology Department on campus was met last Fall. The new instrument contributed to the discovery of aqueous alteration processes in carbonaceous meteorites (Co-I Leshin & Post-Doc Benedix), which provides an important context for understanding extraterrestrial pre-biotic chemistry. This discovery advanced our goal to determine the nature of conditions on the parent bodies of carbonaceous meteorites.

Another part of the ASU commitment to astrobiology was met this year with the hire of geomicrobiologist Ferran Garcia-Pichel, who represents an important interdisciplinary bridge between geology, chemistry and biology/microbiology.

Our goal to understand the origin and evolution of photosynthesis (Co-I Blankenship) was advanced this past year with the development of a model system for early cells constructed using an artificial reaction center and an ATP-synthase enzyme incorporated in a liposome. The model successfully carried out high rates of light-driven ATP synthesis.

Our goal to understand the potential of hydrothermal environments to produce complex pre-biotic organic compounds (Co-I's Holloway & O'Day & Grad Student Vogelsonger) paid off this year with the synthesis of metastable methanol under seafloor hydrothermal conditions, a process predicted by current thermodynamic models.

The Thermal Emission Spectrometer (TES), presently mapping from Mars orbit, discovered several deposits of coarsely crystalline ("specular") hematite (Fe-oxide), which only forms on Earth in the presence of abundant water and usually at elevated temperatures (Co-I Christensen, et al.). This marks an important step in defining potential landing sites for future landed missions for astrobiology. Remote sensing analog studies for Mars were carried out in Death Valley (Co-I Farmer & Post-Doc Moersch) and revealed that a spatial resolution of <100 m/pixel is required to detect evaporite minerals (carbonates and sulfates) using mid-IR. TES maps at 3 km/pixel, indicating the need to fly higher spatial resolution instruments in the future.

HIGHLIGHTS

- A correlation between $\Delta^{17}\text{O}$ of carbonates and the alteration index of CM chondrites was discovered. This finding indicates that these meteorites preserve evidence of evolving fluid compositions on asteroid parent bodies, important for understanding prebiotic conditions for organic synthesis. (Co-I Leshin & Post-Doc Benedix)

- Levels of left-handed amino acid excess in carbonaceous meteorites were explored, and mechanisms for chiral selection effects on asteroid parent bodies were proposed. (Co-I's Cronin & Pizzarello)
- Conditions of deep sea hydrothermal vents were successfully simulated then studied to show the synthesis of primary alcohols (important prebiotic compounds) from inorganic precursors (hydrogen, carbon dioxide and water). (Co-I's Holloway & O'Day & Ph.D Candidate Vogelsonger)
- Enzymes involved in chlorophyll biosynthesis were sequenced, then molecular phylogenetic studies were done, which refined our understanding of the evolution of photosynthesis. (Co-I Blankenship & Collaborators)
- An early cell model was constructed within a liposome, using an artificial reaction center and an ATP-synthase enzyme. This model system successfully carried out high rates of light-driven ATP synthesis. (Co-I Blankenship & Collaborators)
- Several new species of photosynthetic bacteria were isolated and characterized from extreme environments. (Co-I Blankenship)
- A geochemical model was developed, which predicts that Archean (>2.5 billion yrs) ocean salinities were elevated 1.5-2 times the present global ocean average. (Co-I Knauth)
- Dramatic isotopic trends in caliche (carbonates), formed by the arid weathering of basalt (a common Martian rock type), were documented. Biosignatures for fossil life were discovered and preserved in those deposits. (Co-I's Knauth & Farmer)
- An integrated microbiological, geochemical, and paleontological study of travertine hot-spring systems in Yellowstone National Park was conducted, in order to understand the microbial controls on the sedimentology of these systems and the patterns of fossil preservation across major environmental gradients. (Co-I Farmer & Collaborators Des Marais & Fouke)
- Important roles were discovered for exopolymers in microbial fossilization processes in thermal spring environments, which mediate aspects of organic matter preservation, biomineralization and fossilization. (Co-I Farmer & Collaborators Bebout & Visscher)
- Data from the Thermal Emission Spectrometer (TES, presently in Mars orbit) were used to detect deposits of coarse-grained ("specular") hematite, known only to form in the presence of water and typically at elevated temperatures. Such deposits were discovered at several sites, including Terra Meridiani (an ancient paleolake basin), Aram Chaos (a chaos feature formed by outflooding), and the floor of Vallis Marineris (a giant canyon system). These sites represent important landing targets for future Astrobiology missions. (Co-I Christensen & TES team)
- TES data helped to determine that "White Rock" (previously interpreted to be an

evaporite deposit) is more likely to be an intracrater aeolian deposit. (Co-I Christensen & Post-Doc Ruff)

- Based on spectral mapping with TES, it was determined that Mars can be broadly divided into two major compositional provinces: 1) the Southern Highlands, dominated by basalt (an Fe-Mg-silica poor igneous rock) and 2) the Northern Plains, dominated by rocks more enriched in silica. This implies a fundamental process of crustal differentiation that has yet to be understood. However, it could be linked to prolonged aqueous weathering processes on the Northern Plains. (Co-I Christensen, Ph.D Candidate Bandfield & post-doc Hamilton)

- Initial remote sensing analog studies of terrestrial evaporite basins in Death Valley and in the Mono Basin were completed. They showed that carbonates and sulfates are unlikely to be detected from Mars orbit at TES spatial resolution (3 km/pixel). However, with the THEMIS (another mid-IR spectrometer to be flown in 2001), both of these aqueous minerals should be detectable, provided they are present at the surface in amounts exceeding 10-15 wt%. (Co-I Farmer, NRC Post-Doc Moersch, and Master's Candidate Baldrige)

- Geological mapping and site evaluations were completed to identify potential astrobiology landing sites for the Mars 2003 mission. These recommendations were presented to the Mars planning community. (Co-I's Farmer & Greeley & Research Associate Nelson)

- Using data acquired during the Galileo Extended Mission (GEM), several large regions of the surface of Europa were successfully mapped at enhanced spatial resolution and geological models developed to explain the nature and distribution of observed geomorphic features. We also have begun to evaluate the composition of ices exposed at the surface of Europa using multispectral mapping data. Our analyses lend some support for the presence of a subsurface ocean on Europa enriched in magnesium sulfates. (Co-I Greeley & Post-Doc Fagents)

Field Expeditions

Fieldwork for various projects was conducted on the Colorado Plateau of northern Arizona (Knauth and Farmer), at hot springs in Yellowstone National Park (Farmer, Bebout, & Visscher), in Death Valley (Farmer, Post-Doc Moersch, & Masters Candidate Baldrige), the Mono Basin (Moersch & Farmer), at Meteor Crater, Arizona (Kring), in the Cuatro Ciénegas Basin of Central Mexico (Elser, Dowling, Farmer, Garcia-Pichel, PhD Candidate Wade, & Tang), and on the Great Barrier Reef (Heron Island, Australia) (Blankenship).

Cross Team Collaborations

ONGOING

Pizzarello developed collaborations with NASA Ames (Des Marais/Cooper) to study the organic chemistry of carbonaceous meteorites. Farmer established collaborations with the University of Colorado (Jakosky) on site selection strategies for future Mars missions using TES rock abundance data. Farmer and Garcia-Pichel established collaborations with NASA Ames (Des Marais microbial mat team) to work on the molecular biology

(Garcia-Pichel) and microbial taphonomy/meiofaunal grazing (Farmer) of laminated cyanobacterial mats at Guerrerro Negro, Baja. Blankenship developed a collaboration with NASA Ames (Rothchild) to study reactive oxygen enzymes and UV stress in hydrothermal spring mat systems in Yellowstone.

IN THE PLANNING STAGE

Farmer is presently discussing future collaborations, both with Harvard (Knoll) on microbial taphonomy of Late Proterozoic sediments and with Spain (Amils) to study microbial taphonomy of fungal mats along the Tinto River. Knauth has discussed future collaborations with UCLA (Runnegar) concerning work in Death Valley to test the snowball Earth hypothesis.

PUBLICATIONS & ABSTRACTS

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CARNEGIE INSTITUTION OF WASHINGTON

Washington, DC



PRINCIPAL INVESTIGATOR



Sean C.
Solomon

CIW TEAM MEMBERS

Conel Alexander,
*Carnegie Institution Of
Washington*

John Baross,
University Of Washington

Joakim Bebie,
*Carnegie Institution Of
Washington*

James Bischoff,
USGS Menlo Park

Nabil Bector,
*Carnegie Institution Of
Washington*

Alan Boss,
*Carnegie Institution Of
Washington*

Jay Brandes,
University of Texas

R. Paul Butler,
*Carnegie Institution Of
Washington*

Gavin Chan,
Washington University

PROPOSAL EXECUTIVE SUMMARY (1998)

Unifying Intellectual Focus

Our consortium studies the physical, chemical, and biological evolution of hydrothermal systems, including vent complexes associated with ocean ridges, deep aquifers, and other subsurface aqueous environments, both on Earth and on other Solar System and extrasolar bodies. Such diverse systems are important environments for life on Earth and possibly elsewhere in the cosmos. Our principal contribution to the Astrobiology Institute is the experimental, theoretical, and field studies of these environments. We are: (1) experimentally testing ideas relating to the origin and fate of organic molecules in hydrothermal systems; and (2) evaluating several competing hypotheses regarding the possible origin of life in these systems.

Additionally, we are: (1) carrying out observational programs of extrasolar planet detection and theoretical simulations of planet formation; (2) conducting theoretical studies on the formation and evolution of hydrothermal systems on moons and planets; (3) implementing technical development of apparatus appropriate for physical, chemical, and biological studies at rigorously controlled high-pressure hydrothermal conditions; and (4) engaging in field studies for the recovery and preservation of microorganisms from hydrothermal systems.

Motivation

The most exciting opportunities afforded NAI members are the new integrated approaches and collaborative projects that are arising from interactions among the Institute research teams. We are taking advantage of these opportunities. Consequently, we structured our consortium in a way that is both clearly focused on a specific and fundamental scientific question (the role of hydrothermal systems in biological evolution), and unusually broad (integrating the experimental, theoretical, and field studies of leaders in astrophysics, geochemistry, oceanography, and biology). Thus, we hope to make significant progress toward understanding one important aspect of astrobiology, while serving as a dynamic, interactive center for other NAI efforts.

Rationale

The traditional view of life's origin on Earth has focused on processes near the photic

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zone at the ocean-atmosphere interface, where ionizing radiation provides energy for prebiotic organic synthesis. In the context of astrobiology, this origin paradigm restricts the initial "habitable zone" around stars to planets and moons with surface water. According to this view, subsequent adaptations on Earth, and possibly elsewhere, led to expansion of the biosphere into subsurface habitats.

An alternative hypothesis is that life-forming processes may also occur in subsurface, hydrothermal environments at the water-mineral interface. This hypothesis, that life on Earth originated from oxidation-reduction reactions in deep hydrothermal zones (perhaps at or near ocean ridge systems), opens exciting possibilities for astrobiological research. If a subsurface, high-pressure origin of life is possible, then the initial habitable zone around stars is greatly expanded to aqueous environments where redox reactions can be driven by thermal energy.

Four lines of evidence lend credibility to the hydrothermal origins hypothesis: (1) numerous recent discoveries of high-pressure life, especially lithotrophic prokaryotes, suggest that hydrothermal environments support abundant life; (2) models of the Earth's formation postulate large, surface-sterilizing impacts as recently as 3.8 billion years ago, and deep hydrothermal zones may have insulated life from these insults; (3) studies of molecular phylogeny reveal that thermophilic microbes are perhaps the closest living relatives of the last universal common ancestor; and (4) hydrothermal organic synthesis experiments reveal unexpectedly facile synthetic pathways. Whether or not life originated in a subsurface hydrothermal zone, these lines of evidence (coupled with the assumed widespread distribution of such environments in our Solar System and elsewhere) point to the need and opportunity for an intense study of the physical, chemical, and biological characteristics of hydrothermal systems.

Research Plan

Our research activities explore the physical, chemical, and biological evolution of hydrothermal systems from these complementary fronts:

- We model planetary formation, and we detect and characterize extrasolar planets, in an effort to understand the range of objects that develop hydrothermal systems and to understand the distribution of volatiles (especially water) within those objects.

Kenneth Chick,
*Carnegie Institution Of
Washington*

I-Ming Chou,
USGS Reston

George Cody,
*Carnegie Institution Of
Washington*

David Emerson,
George Mason University

Timothy Filley,
*Carnegie Institution Of
Washington*

Marilyn Fogel,
*Carnegie Institution Of
Washington*

Erik Hauri,
*Carnegie Institution Of
Washington*

Robert Hazen,
*Carnegie Institution Of
Washington*

Russell Hemley,
*Carnegie Institution Of
Washington*

Satoshi Inaba,
*Carnegie Institution Of
Washington*

Catherine Johnson,
*Carnegie Institution Of
Washington*

Stephen Kortenkamp,
*Carnegie Institution Of
Washington*

Kono Lemke,
Stanford University

Patrick McGovern,
*Carnegie Institution Of
Washington*

D. Arcy Meyer,
Washington University

Harold Morowitz,
George Mason University

David Ross,
USGS Menlo Park

James Scott,
*Carnegie Institution Of
Washington*

Anurag Sharma,
*Carnegie Institution Of
Washington*

Everett Shock,
Washington University

Sean Solomon,
*Carnegie Institution Of
Washington*

Harri Vanhala,
*Carnegie Institution Of
Washington*

Jianhua Wang,
*Carnegie Institution Of
Washington*

George Wetherill,
*Carnegie Institution Of
Washington*

Hatten Yoder,
*Carnegie Institution Of
Washington*

Susan Ziegler,
*Carnegie Institution Of
Washington*

Misha Zolotov,
Washington University

- We investigate the processes by which hydrothermal systems evolve on planets and other bodies. Under what circumstances do hydrothermal systems form, and what are their physical and chemical characteristics?

- We study geochemical processes in hydrothermal systems, especially those that lead to abiotic organic synthesis. We focus in particular on the role of mineral catalysis in these systems.

- We consider the origin and evolution of biological entities in hydrothermal systems through studies of the biochemistry of contemporary hydrothermal organisms.

A complete understanding of hydrothermal systems and their role in life's origins will require dramatic advances on all of these fronts, as well as extensive and challenging integration of these topics. During the first five years of NAI, we anticipate significant progress in each of these four research areas, as well as increased attention to the interfaces among these theoretical, experimental, and field studies.

Training, Education, and Public Outreach

As members of the NASA Astrobiology Institute, we are committed to a dynamic, sustained program of education and public outreach at the K-12, undergraduate, graduate, and postgraduate levels, including in-service training for K-12 teachers. Our emphasis throughout is on enhancing opportunities for broadly integrated science learning. These programs build on widely acknowledged programs, including the First Light Science School, the Carnegie Academy for Science Education (CASE), Carnegie Institution Summer Intern Program, and the Capital Science Lecture Series. These named programs are all now in place at the Carnegie Institution of Washington, and they continue to be developed in strict accordance with NASA education and outreach guidelines. In summary, our NAI education and outreach programs include the following:

- We are introducing NAI themes to CASE, which is utilizing these educational materials to retrain more than 1000 public elementary school teachers from the District of Columbia and at regional workshops throughout the country during the first 5 years of NAI. A professional development video featuring classroom instruction with children will be created and made available for training educators within their region.

- We are introducing NAI themes as we develop and distribute educational packets for grades 2-7 through the Carnegie Institution's First Light Science School, which targets children from predominantly minority urban areas of D.C. These materials will be available both through a Carnegie/NAI website and in printed editions for national distribution.

- We support approximately 12 NAI Summer Interns (undergraduate science majors and advanced high school students) per year.

- We support approximately 10 NAI Postdoctoral and Predoctoral Fellows per year.

- We highlight NAI themes through the Capital Science Lecture Series, a highly successful public lecture venue sponsored by the Carnegie Institution.

- We continue Carnegie's program of national public outreach through general interest publications on NAI themes.

Management Plan

All investigators and collaborators have clearly defined roles and responsibilities. Consortium activities are managed by an Executive Committee, consisting of representatives from each of the geographic nodes in the consortium and chaired by the Principal Investigator. An extensive plan for multi-institutional cooperation and coordination includes regular visits of senior staff to partner institutions, and more extended exchanges of students, postdoctoral scholars, and other staff. Through use of the internet and the World Wide Web we are making extensive and continuing use of electronic communication. Beyond frequent communication among individuals, we plan the development of web-based instructional and outreach materials, full utilization of electronic communication to enable access to data archives, and (in the longer term) remote interaction with ongoing experiments.

Proposed Institutional Commitment

The Carnegie Institution of Washington has made a substantial commitment of resources to this astrobiological initiative. Regarding NAI project personnel, the institution has provided: (1) full salary for the Investigators, all twelve members of the senior research staff; (2) 50% of the stipends of all Postdoctoral Research Associates, who will be working full time on research tasks described in this proposal; (3) full support of 12 college and local high-school students, who will work on astrobiological research projects as summer interns in our laboratories; and (4) full support of all technicians and computer staff, who are responsible for the maintenance and operation of the facilities to be used in NAI research. An array of laboratory instrumentation with an aggregate value in excess of \$5 million will be made freely available to research in support of Astrobiology Institute objectives. The Carnegie Institution has also made a significant financial commitment to the development and implementation of programs in science education (CASE), which will provide a platform for the effective preparation and distribution of NAI-related educational materials. Significant contributions of salary support and facilities have also been made by each of the partner institutions in our consortium.

Innovation and Distinguishing Features

Our consortium is distinguished by three innovative features:

- It features unusual scientific breadth. We closely integrate experimental, theoretical, and field studies by experts in astrophysics, biology, geochemistry, mineralogy, oceanography, petrology, and planetology.
- It focuses this broad expertise on the evolution of hydrothermal systems, a key need and opportunity in astrobiology.
- It expands the rich and diverse array of training, education, and outreach programs now in progress, both at the Carnegie Institution of Washington and at our partner institutions. These programs reach students at all levels (K-12, undergraduate, graduate, and postgraduate), as well as teacher in-service training, and the general public.

Roadmap Objectives

#6
Microbial Ecology

#7
Extremes of Life

SUMMARY

The opportunity to develop these and other collaborative ties within the broadly interdisciplinary NASA Astrobiology Institute, in the context of our existing dynamic research and educational endeavors, provides our primary motivation for this proposal. We participate in this unique program with enthusiasm and excitement.

Project

Biological Studies of Hydrothermal Systems

Senior Project Investigator(s):

John A. Baross, David Emerson,
Marilyn L. Fogel

ACCOMPLISHMENTS

Task 1.

Field Studies and Laboratory Characterization of Hydrothermal Vent Microbes (Baross)

We have completed the characterization of unique seafloor hyperthermophiles. One of the strains was found to oxidize acetate anaerobically using Fe(III) as the electron acceptor. In this process, the Fe(III) is converted to magnetite. This is the first hyperthermophilic archaea to show this physiology and the first unique subsurface hyperthermophile isolated. This organism is a new genus within the archaeal family Thermococcales and one of the deepest roots in the Archaea phylogenetic tree. A manuscript describing this organism is being prepared for publication in *Science*.

The molecular-phylogenetic analyses of the subsurface microbial communities from the 1998 deep-sea eruption at Axial Volcano, Juan de Fuca Ridge, has been completed for samples collected in 1998 and 1999. There is a huge diversity of both archaea and bacteria in these subsurface water samples, most of which are not related to any known cultured organisms. Some of the archaeal sequences form clusters that are not related to any reported environmental sequences. We are in the process of trying to decipher the physiological characteristics of these uncultured archaea using molecular methods and novel culturing procedures.

A unique thermophilic group of bacteria was isolated from Axial Volcano subsurface fluids. One of these strains grows in extremely low levels of organic material or with carbon dioxide and hydrogen. These organisms produce a significant amount of extracellular polysaccharides used in biofilm formation. These organisms are unrelated to any known cultured bacteria and could be a new family of thermophilic bacteria. A manuscript describing this subsurface diversity is being prepared. We also will return to Axial in July 2000 to obtain additional samples.

A molecular probe has been designed to detect the NIF (nitrogen fixation) gene in archaea and has been used successfully to detect the NIF gene in hyperthermophiles

from subseafloor environments. We are currently in the process of cloning and expressing these archaeal NIF genes.

A preliminary description of the microbial ecology of active sulfide chimneys has been completed using a combination of analyses. Analytic techniques include environmental scanning electron microscopy, EDAX (energy dispersive X-ray analysis), epifluorescence microscopy, and FISH (fluorescence *in situ* hybridization). These analyses provide quantitative estimates of total cell numbers, the abundance of specific phylogenetic domains, and phylogenetic analyses of the microbial communities using 16S rRNA gene sequences. These data show that only archaea inhabit the high temperature zones of the sulfide. There is evidence that intact microbial colonies exist in mineral zones at temperatures greater than 150°C. A manuscript is being prepared for *Science*.

Task 2.

Stable Isotope Studies of Hydrothermal Organisms (Emerson, Fogel)

We have started investigations into the stable isotopic compositions of hydrothermal vent organisms grown in culture at the American Type Culture Collection in Manassas, Virginia. Isotopic experiments were designed to test if we could detect differences in central metabolic pathways through analyses of carbon isotopic compositions of individual amino acids. For these experiments, a variety of Archaeobacteria and Eubacteria with different metabolic pathways were cultured and analyzed. In addition, equipment for culturing microorganisms was purchased and installed at the Carnegie Geophysical Laboratory to provide supporting microbial material for isotopic analyses.

Isotopic compositions of amino acids can be used to determine branch points in metabolic synthesis of amino acids. In addition, we are comparing metabolic pathways in different organisms from various positions in the Universal Tree by investigating isotopic fractionation of particular amino acids relative to others. For example, glutamic acid and aspartic acid are both synthesized by the TCA (Krebs) cycle. The TCA cycle operates either in the forward or the reverse direction in microbes, depending on the organism or the growth conditions. It is often assumed that the enzymes responsible for synthesis of amino acids are fairly conservative. We have found that we are able to distinguish different pathways and different enzymes with differential fractionation of carbon isotopes in amino acids. The long term goal of this research is to be able to link known physiology to a particular carbon isotopic fractionation.

In concert with this work, we have been investigating the stability of proteins in geochemical environments. With sensitive protein assays, we have determined that proteins from primary photosynthetic producers turn over rapidly and the original isotopic composition is almost completely scrambled. Experiments are beginning with new methodology to be able to recognize and assign chemical structures and molecular weight to complex diagenetic organic matter. To this end, we have used stable isotopes as tracers and are developing methodology for determining the D- and L-enantiomer ratios and isotopic ratios of geochemically-derived amino acids. Samples from crustal fluids will be analyzed for D- and L-amino acids and their corresponding isotopic compositions to provide an overall framework for abiotic or biological synthesis of organic matter in hydrothermal environments.

Roadmap Objectives

- #1 Sources of Organics on Earth
- #2 Origin of Life's Cellular Components
- #7 Extremes of Life
- #8 Past and Present Life on Mars
- #9 Life's Precursors & Habitats in the Outer Solar System
- #11 Origin of Habitable Planets
- #13 Extrasolar Biomarkers

Field Expeditions

1. Axial Volcano, Juan de Fuca Ridge (July 1999); NOAA/SeaGrant sponsored cruise. J. Baross with students J. Huber and J. Kaye.

Archaeal and bacterial diversity in the hot subseafloor environment were studied using molecular phylogeny methods. Research included isolation and characterization of hyperthermophilic archaea and bacteria.

2. Axial Volcano, Juan de Fuca Ridge (July 2000); NOAA/SeaGrant sponsored cruise. J. Baross with students J. Huber and M. Mehta.

Again, archaeal and bacterial diversity in the hot subsurface environment were studied using molecular phylogeny methods. Research again included isolation and characterization of novel hyperthermophilic archaea and bacteria. Incidence and characteristics of the NIF (nitrogen fixation) gene were also studied in subseafloor microbial communities.

3. Endeavour vent field, Juan de Fuca Ridge (June 2000; September 2000).

Chief-scientists: J. Delaney and J. Kelly.

NAI provides support to participate on these cruises with participants J. Baross and students J. Kaye and M. Schrenk.

Diversity of hyperthermophilic archaea and halophilic bacteria and archaea from diffuse-flow vents and sulfides were studied using molecular and culturing methods.

Cross Team Collaborations

Fogel and Scott are active collaborators on the NAI team at JPL.

Project

Hydrothermal Organic Synthesis

Senior Project Investigator(s):

George D. Cody, Robert M. Hazen,
James L. Bischoff

ACCOMPLISHMENTS

Experiments designed to explore the potential hydrothermal roots of primitive biochemistry are being done by researchers at both the Carnegie Institution of Washington (CIW) and the U.S. Geological Survey (USGS) under the auspices of the NASA Astrobiology Institute. The principle task areas within this program include: (1) the experimentally constrained analysis of the potential for development of primitive metabolism under conditions that mimic those of deep sea hydrothermal vents (CIW); (2) the relationship between mineral surface topology and the development of homochirality (CIW); and (3) mechanistic studies on the formation of amino acids, oligopeptides, and polypeptides under hydrothermal conditions (USGS). Integral to the successful com-

pletion of these task areas is the development of state-of-the-art experimental methods that exploit flow-through hydrothermal reactors, hydrothermal diamond anvil cell equipment, as well as the development of micro-analytical methods enabling the interrogation of organic reaction progress in-situ, i.e., under conditions of high temperature and pressure. The first year of this project focused on the assembly, testing, and optimization of these instruments, while the second year has focused on implementation of these methods to address astrobiological research. (See individual task area reports below).

Task 1.

Experimental Search for the Geochemical Roots of Biochemistry (Cody).

This task area focuses on establishing the roots of metabolic chemistry potentially intrinsic to the geochemical environments of deep sea hydrothermal vents. Over the past year, we have been successful on a number of fronts. Cody et al. have established a potential entry point into the reductive TCA cycle, utilizing transition metal sulfides and reduced carbon bearing fluids. This pathway (named the hydrothermal redox pathway) is not used in any extant organisms, but it may have been the ignition point for primary metabolism. Cody et al. have begun to explore the potential role of organometallic phases that may be intrinsic to hydrothermal systems as sources of primitive biological energy conversion functionality. The potentially critical catalytic role of such species may have been a crucial link between geochemistry and biochemistry at the point of life's emergence. Sharma et al. have developed new techniques for *in situ* study of biochemically relevant reactions at elevated temperatures and pressure utilizing a hydrothermal diamond anvil cell (DAC). Loading nanoliter quantities of sample within the DAC, Sharma et al. have combined optical microscopy and Raman spectroscopy allowing for the analysis of complex reaction kinetics at high T (temperature) and P (pressure). Additionally, procedures have been developed to extract organic samples from the DAC following reaction, followed by analysis using GC/MS (gas chromatography/mass spectrometry), allowing us to compare the *in situ* data with those previously derived using sealed reactor experiments. Filley et al. have begun studying carbonylation reactions using the high T and P flow reactor. Preliminary results point to this method as being an effective tool for deconvolution of the reaction mechanisms. Integrating all of this, we find ourselves within one reaction of demonstrating a purely geochemical carbon fixation pathway that closely mimics the combined acetyl-CoA and reductive TCA pathways. Whether this pathway was the focal point for emergence of primary metabolism remains to be established, but the facility of these reactions makes a strong case for such a pathway lying at the pre-enzymatic roots of biochemistry.

Task 2.

The Possible Role of Mineral Surfaces in Prebiotic Chiral Selectivity (Hazen).

Life employs left-handed amino acids almost exclusively, with the notable exception of some bacterial cell wall components. The emergence of such chiral selectivity in life from a supposedly racemic prebiotic amino acid pool remains a central problem in origin of life research. In abiotic synthesis, optically active products may be synthesized if either the substrate, reagent, or catalyst is optically active. No plausible scenario has accounted for the production of a large chiral excess of substrate or reagent in prebiotic amino acid and peptide syntheses. Minerals can provide a simple solution to this problem, because most common minerals feature surfaces that are inherently chirally

selective. Quartz, which occurs in both right-handed and left-handed structural variants, has long been recognized in this regard. However, little attention has been focused on the majority of centrosymmetric minerals, including rock-forming silicates, carbonates, sulphates, and phosphates. These minerals commonly possess pairs of crystal faces with surface structures as mirror images of each other. Such surfaces are ideally suited to select and concentrate L- and R- centers in molecules, such as amino acids. Left- and right-handed mineral surfaces occur in approximately equal numbers, so they cannot produce chiral selection on a global scale. However, the origin of life was a local event, not a global one. The first life form arose at a specific place and time. The origin of life is, in some respects, analogous to the formation of a crystal. In each case, nucleation and growth are essential and independent steps. It is reasonable to assume that nucleation of life (the self-organization of molecules, perhaps on a mineral surface) is rare, perhaps even a singular event. However, once this proto-life formed, it likely came to become dominant. Experiments are currently being conducted to test for chiral selectivity on carefully selected minerals.

Task 3.

Amino Acid Synthesis Under Hydrothermal Conditions (Bischoff).

An inherent mark of past studies on hydrothermal amino acid synthesis is the presence of formaldehyde in the short list of primitive starting materials. Formaldehyde is quite easily oxidized, however, and a fundamental notion can be developed questioning its presence in the prebiotic atmosphere at meaningful levels. With that view in mind, and in response to recent literature accounts describing the rapid conversion of CO₂ to formic acid on common mineral surfaces in both hydrothermal and sunlight-promoted settings, the USGS team has begun studies in hydrothermal media excluding formaldehyde. The experiments conducted at 210°C over 2-3 hr periods included cyanide (CN⁻). This is also considered a key primitive starting material, though under the prevailing conditions, it is rapidly converted to a formic acid salt. Our study shows that amino acids are formed rapidly in the absence of formaldehyde with the product mixture containing glycine, racemic alanine, and racemic aspartic acid. (Note that the racemic quality of the acids assures that no contamination has taken place.) While further work is underway to confirm these results, this finding appears to put into question the long-held view that the formaldehyde based Strecker synthesis is the basis of prebiotic formation of amino acids. Another experimental series showed that amino acids were not produced when the feed contained formaldehyde but no cyanide. However, when the same system was spiked with glycine, significant quantities of racemic alanine, serine, and aspartic acid were formed. This work is continuing. A preliminary proposition stemming from these results is that the higher acids are formed from the parent glycine. Such a scenario, if confirmed, would immediately explain why almost all of the essential amino acids are alpha-substituted.

HIGHLIGHTS

- We have established a potential entry point into the reductive TCA cycle utilizing transition metal sulfides and reduced carbon bearing fluids. This pathway, named the hydrothermal redox pathway, is not used in any extant organisms, but it may have been the ignition point for primary metabolism. We have established that a broad range of potentially naturally-occurring mineral sulfides are capable of promoting reactions that

Year 2

mimic aspects of reactive centers in enzymes controlling primary metabolism. We have developed state-of-the-art instruments and methods for analysis of organic reaction networks under extreme conditions.

- Our preliminary results on amino acid synthesis appear to question the common view that prebiotic synthesis required formaldehyde, a result in line with the notion that formaldehyde may not have been present in significant quantities in the prebiotic world. Our findings may provide insight into the fact that most of the essential amino acids are alpha substituted, a feature of the early life-related chemistry that remains unexplained.

Cross Team Collaborations

In the course of this research, we have forged collaborations with other researchers within and outside of the NAI, including scientists at the USGS in Reston and NAI members at ASU and PSU.

A member of one of the USGS teams (DSR) is also a member of the Europa Focus Group, currently being organized across NAI.

Studies of Organic Matter and Water in Meteorites

Project

Senior Project Investigator(s):

Conel M. O'D. Alexander, Nabil Bector, George D. Cody, Erik H. Hauri

ACCOMPLISHMENTS

The organic material in primitive chondritic meteorites has attracted considerable attention, not only because it retains a record of synthesis in the interstellar medium (ISM), but also because L- enantiomer excesses have been reported in meteoritic amino acids. If the meteorite organics are typical of the material accreted by the prebiotic Earth, this may explain the homochirality of terrestrial life. The ISM origin of some or all the meteorite organics suggests the intriguing possibility that this or similar material is a source of complex prebiotic organics, not just in our solar system but in all solar systems. The amino acids, nucleic acids, and other soluble organics found in the meteorites probably formed by hydrolysis of the more abundant macromolecular material, with its enigmatic structure and origin. We are currently using a range of techniques to determine the structure of this macromolecular material. From this, we hope to learn how it formed and how it would break down under various conditions to produce important, complex prebiotic molecules.

The Martian meteorites (SNCs) are our only sample of another planet. Early conditions on Mars may well have been conducive to the development of life. However, the surface of Mars is now an arid and inhospitable environment for life. The key to understanding how long conditions were conducive to life and whether life might still persist at depth on Mars is the evolution of water. Martian meteorites do contain water bear-

Roadmap Objectives

#1

Sources of Organics on Earth

#8

Past and Present Life on Mars

#9

Life's Precursors & Habitats in the Outer Solar System

#11

Origin of Habitable Planets

ing phases. The water in these phases is typically enriched in deuterium. The current Martian atmosphere is also enriched in deuterium as a result of the loss of water to space. If the deuterium-rich water in the oldest Martian meteorite (ALH 84001 -- 4.0 Ga) reflects the composition of the ancient Martian atmosphere, Mars had lost most of its water very early, leaving little time for life to evolve. However, there are processes associated with the intense shock experienced by most Martian meteorites that may have produced the deuterium enrichments. We are trying to determine which of the two possible explanations for the deuterium enrichment is the correct one.

Task 1.

Sources of Extraterrestrial Water in Martian Meteorites (Boctor, Alexander, Hauri)

We continued our investigations of H isotope compositions and the sources of extraterrestrial water in Martian meteorites. We previously investigated these Martian meteorites: ALH 84001 and EETA 79001. We detected an extraterrestrial hydrogen isotope signature for water in four phases in these meteorites: (1) carbonate and phosphate minerals; and (2) impact-melted feldspathic and mafic glass. The glasses in these meteorites have the highest δD values (+969 to +2901 ‰). A weak positive correlation was observed between the δD values and the intensity of shock. For example, mafic glasses (shock pressure > 60 GPa) had higher δD values than feldspathic glasses (shock pressure of 40 GPa). We interpreted the hydrogen isotope data by one of two hypotheses: (1) the glasses reacted with a water reservoir on Mars that equilibrated with a highly fractionated Martian atmosphere; or (2) impact induced hydrogen loss from the glasses by devolatilization.

We extended our H isotope investigations to these Martian meteorites: (1) ALHA 77005, a cumulate lherzolitic shergottite; and (2) chassigny, the only known Martian dunite. The olivine in both minerals contains trapped melt inclusions that may have preserved the hydrogen isotope composition of their parent magmas or were less affected by shock than the glasses produced by impact melting. The glass in the melt inclusions from ALHA 77005 has low δD values (-18 to +304 ‰) and very low water contents (1.1×10^{-4} to 7.4×10^{-5} wt %). Feldspathic and mafic impact melted glass showed higher δD (1301 to 3031 ‰) and much higher water contents (8.1×10^{-3} to 5.2×10^{-2} wt %). The data suggest that the source of water in the impact-melted glasses is different from that of the magmatic glass in the melt inclusions. We suggest that surface water with an approximate Martian atmosphere signature or a less fractionated subsurface water was incorporated in the glass during impact melting. During decompression, as water solubilities in the melts decreased further, D enrichment by devolatilization of H from the melts may have occurred. Shock experiments have shown significant loss of water by impact and that the water remaining in shocked samples became more enriched in D. The magnitude of fractionation is related to the degree of melting.

Melt inclusion glass in chassigny has low δD values (+90 to +223 ‰) that are comparable to melt inclusion glasses in ALHA 77005. No impact melted glasses were observed in chassigny. Plagioclase, which shows variable solid state shock effects, yielded δD values (+ 90 to + 688 ‰) much lower than those we observed in impact melted feldspathic glass in other Martian meteorites. These values may be attributed in part to mixing with low D terrestrial contamination.

Task 2.

Macromolecular Organic Matter in Carbonaceous Chondrites (Cody, Alexander).

This project focuses on determining quantitatively, albeit statistically, the organic structure of the macromolecular phase(s) in carbonaceous chondrites. Using a new demineralization method developed in house, we are now able to obtain very high quality ^{13}C solid-state NMR spectra of the macromolecular organic material isolated from meteorites. This method utilizes both cross polarization (from hydrogen, or CP) and single-pulse excitation (carbon with proton decoupling, or SPE). We have obtained the first SPE spectra for the Murchison macromolecular organic matter using realistic acquisition parameters. The critical aspect of these data is through the comparison CP and SPE spectra. CP methods essentially reveal that the carbon chemistry proximal to hydrogen, whereas SPE reveals all of the carbon (i.e., the spectra are not dependent on proximity to hydrogen). If there exists within the macromolecular phase large carbonaceous domains devoid of hydrogen, (e.g., fulleroid- or graphitoid-like domains), one should see this manifested by significant differences between CP and SPE. Remarkably, minimal differences are observed in the various organic functional groups detected using either NMR experiment. Previous results employing pyrolytic methods have suggested that there exists a very-high-molecular-weight component of the insoluble macromolecular fraction (possibly 25-40 % of the carbon) that is likely to be hydrogen deficient (perhaps graphitoid or fulleroid in structure). The spectroscopic results above bring this interpretation into question. In addition to carbon, we have acquired very-high-quality solid-state spectra of ^1H employing an extremely fast magic-angle-spinning probe (frequency 1,800,000 RPM). These data provide the first ever determination of hydrogen speciation within the macromolecular phase of the Murchison meteorite. We are now in the process of synthesizing these data into a self-consistent molecular model of organic functionality that will serve as a basis for understanding organosynthesis in the presolar nebula.

Cross Team Collaborations

The research described above under Task 1 (Sources of Extraterrestrial Water in Martian Meteorites) constitutes CIW's connection to a new cross-NAI "Focus Group on Martian Meteorites", led by Dr. David McKay of NASA Johnson Space Center.

The research described above under Task 2 (Macromolecular Organic matter in Carbonaceous Chondrites) constitutes CIW's connection to a new cross-NAI effort to develop an "Extraterrestrial Macromolecular Characterization Focus Group." This group involves core members from CIW, Dr. Robert Minard of the Penn State NAI Team, and Dr. Gene MacDonald of the JPL NAI team, as well as a number of other NAI members.

Roadmap Objectives

#8

Past and Present Life
on Mars

#9

Life's Precursors &
Habitats in the Outer
Solar System

#11

Origin of Habitable
Planets

#12

Effects of Climate &
Geology on Habitability

Project

Studies in Planetary Formation and Evolution

Senior Project Investigator(s):

Sean C. Solomon, Alan P. Boss, R. Paul
Butler, George W. Wetherill

ACCOMPLISHMENTS

Task 1.

Detection and Characterization of Extrasolar Planets (Butler).

Over the last year, our collaboration at Lick and Keck Observatories (Geoff Marcy - UC Berkeley; Steve Vogt - UC Santa Cruz; Debra Fischer - UC Berkeley) has produced more than one extrasolar planet a month, including these findings: (1) the only known multiple-planet system orbiting a Sun-like star (upsilon Andromedae); (2) the only known transit planet (HD 209458); and (3) the first two sub-Saturn-mass planets (79 Ceti and HD 46375). These discoveries have helped push the total number of known extrasolar planets to over forty. We are currently focused on improving measurement precision so that we will be able to detect planets down to the one Neptune-mass range. Along with Steve Sackett (Carnegie Observatories), we are putting together a southern hemisphere planet search on the 6.5-m Magellan I telescope, planned to begin in 2001. Together with our current 200-star survey on the 3.9-m Anglo-Australian Telescope (AAT), the Magellan survey will extend our southern hemisphere search to 800 stars. Along with our surveys on the Lick 3-m and the Keck 10-m, the AAT and Magellan projects will allow us to observe the 2000 nearest Sun-like stars. Our long term goals include: (1) discovering Jupiter and Saturn-like planets beyond 4 AU to compare with our own solar system to provide targets for follow up with new techniques such as interferometry and IR imaging; and (2) finding enough planets to generate statistically meaningful distributions of planet mass, orbital distance, eccentricity, and metallicity of planet-bearing stars. These distributions will be required to constrain theories of planet formation and evolution.

Task 2.

Formation of Gas-Giant Planets (Boss).

Gas giant planets have been detected in orbit around an increasing number of nearby stars. Two theories have been advanced for the formation of such planets: core accretion and disk instability. Core accretion, the generally accepted mechanism, requires several million years or more to form a gas giant planet in a protoplanetary disk like the solar nebula. Disk instability, on the other hand, can form a gas-giant protoplanet in a few hundred years. However, disk instability has previously been thought to be important only in relatively massive disks. Co-I Boss has calculated a series of new three-dimensional hydrodynamical models. These show that disk instability could form Jupiter-mass clumps, even in a disk with a mass (0.091 solar masses within 20 AU) low enough to be in the range inferred for the solar nebula. The clumps form with initially eccentric orbits, and their survival will depend on their ability to contract to higher densities before they can be tidally disrupted at successive periastrons. Because the disk

mass in these models is comparable to that apparently required for the core accretion mechanism to operate, Boss's models imply that disk instability could obviate the core accretion mechanism (in the solar nebula and elsewhere), possibly with important consequences for the formation of terrestrial planets in these systems.

Task 3.

Formation of Earth-like Planets (Wetherill).

The long-term goal of our research is to contribute to the understanding of the extent to which globally habitable planets like Earth are abundant or rare. Ultimately, the answer to this question will require superior observations of other planetary systems. Our work seeks to contribute to providing a theoretical framework for the planning and interpretation of these observations. Our immediate goal is to help solve some vexing problems that now stand in the way of achieving this.

These problems have recently come to light while trying to understand formation of our own planetary system. This difficulty could be a consequence of the rarity of such systems and the very special circumstances required to form them, circumstances that would be considered ad hoc if they were suggested. Our more optimistic assumption is that our theoretical tools have advanced only far enough to display previously unrecognized difficulties, but not far enough to resolve them.

These difficulties arise as a consequence of the powerful gravitational effects of the giant planets, Jupiter and Saturn. After these planets became present, they played a dominant role in controlling the growth of all other bodies in the solar system. Until recently, it was assumed that the growth of Jupiter could be delayed by as much as 10 million years, long enough to permit inner solar system bodies to grow from dust to bodies about the size of Mars without undue interference by the giant planets. After reaching this size, the effects of the giant planets would not have been sufficient to keep the Earth and its partners from continuing on to their present size.

Now, on both observational and theoretical grounds, it appears likely that if Jupiter (and Saturn) formed in the conventional manner, these giant planets would rapidly drift into the Sun during their formation. Our colleague, Alan Boss, has proposed and quantitatively developed a very promising alternative way to form the giant planets. In this theory, the giant planets grow far more rapidly, as a result of massive gravitational instabilities in the pre-solar disk of dust and gas. An important feature of this model is that the giant planets could finish growing before they drifted away. Then, if the gas were removed soon enough, possibly after about 1 million years, these planets might drift only to their present distance. As is the case for our own theoretical work, the details of this drifting phenomenon are still under development by qualified scientists.

The conventional theoretical techniques for calculating the growth of terrestrial planets from very small planetesimals do not work in the presence of giant planet perturbations that would occur in this new model. During the past year, we have developed the theory and computer algorithms necessary to make such calculations. Using possible assumptions regarding the drift process, we find that the Earth and other terrestrial planets are likely to grow large enough to withstand the gravitational effects of the giant planets as quickly as 10,000 to 100,000 years. This is not the consequence of the well

Roadmap Objectives

#1

Sources of Organics on Earth

#5

Linking Planetary & Biological Evolution

#9

Life's Precursors & Habitats in the Outer Solar System

established process of runaway growth. Rather, it is a new phenomenon. This involves different physical processes with a similar result and the same time scale, despite fundamental differences in the gravitational processes responsible. At present, we are examining the consequences of this mechanism for the formation of asteroids. We will continue this study for the coming year. Because of their greater distance from the Sun, as well as their greater proximity to Jupiter, asteroids will certainly not be able to grow as large as the terrestrial planets, and they do not. We hope that in using some combination of other people's drift models and our planetesimal models, it will prove possible to fit them into this picture. If not, other ways will need to be found to resolve these problems. Such solutions are essential if the goals of this Institute are to be achieved.

Task 4.

Evolution of Planetary Water (Solomon)

One of the long-term goals of this NAI project is to assess the likelihood and timing, as well as the physical and chemical environments, of hydrothermal systems on solar system objects other than Earth. Our efforts on this project during the past year have focused on Mars, primarily because of the influx of important new data from the Mars Global Surveyor (MGS) mission. As a result of measurements by the Mars Orbital Laser Altimeter (MOLA), we now have much-improved estimates for the volume of water ice at the Martian poles. We have also explored the conditions (local heat flux, plus the mix of sediment and ice in polar deposits) under which basal melting might occur beneath the polar ice. New gravity measurements by MGS have revealed negative gravity anomalies in the northern plains of Mars. Because these gravity anomalies have no expression in MOLA topography, subsurface mass deficiencies are implied. On the basis of the linear form of the anomalies and their location downslope from channel features at higher elevations, we have proposed that the anomalies represent ancient buried channels now filled with sediment of lower density than average crustal material. An implication of this hypothesis is that the volume of water transported from southern highlands to northern lowlands over Martian history has been much greater than heretofore appreciated.

Project

Theoretical Studies of Hydrothermal Synthesis Reactions

Senior Project Investigator(s):

Harold J. Morowitz, Everett L. Shock

ACCOMPLISHMENTS

Task 1.

Energetics of Hydrothermal Ecosystems (Shock).

Research conducted during the past year on this project falls into three categories: (1) efforts to understand the energetics of terrestrial hydrothermal ecosystems, as well as the energetic demands of individual thermophilic and hyperthermophilic microorganisms; (2) predictions of the extent of the subsurface biosphere on the Earth; and (3) connecting the constraints on organic synthesis in hydrothermal and volcanic systems

with those extracted from theoretical models of planetary evolution. We have made progress in understanding what generates disequilibrium states in geochemical systems, the delicate interplay between thermodynamics and kinetics. These disequilibrium states are sources of energy for subsurface organisms. There can be no doubt that the surface of a planet can be irrelevant in determining the potential for life. We are illuminating the connection between the quantities of geochemical energy and biomass that can be supported. In addition, new models of organic synthesis on meteorite parent bodies and icy satellites are revealing the complementary roles of volcanic, impact, and hydrothermal processes in generating and altering the abiotic organic inventory of the solar system.

Plans for the coming year call for completion of several papers that will constitute Gavin Chan's Ph.D. thesis. These will explore the relations between geochemistry and microbial energetics throughout the Earth's crust. Plans also include new models of organic synthesis during formation of the solar nebula to be conducted by Misha Zolotov.

Task 2.

Theoretical Foundation for Organic Synthesis Experiments (Morowitz).

We have developed a search strategy for the Beilstein online database (3.5 million chemical compounds) to look for the subset that would have been generated from aqueous CO₂ and reductants in the absence of enzymes. These sequential steps characterize our search strategy: (1) confine the search to compounds with between one and six carbon atoms, on the assumption that we are building up from one-carbon compounds; (2) confine the set to a water partition in a water-oil system; (3) select for highly-oxidized molecules starting from CO₂; (4) select for low heats of combustion to be near the starting state of carbons; and (5) eliminate unstable peroxides and carbon triple bonds. This search yields a subset of 153 molecules, containing all 11 molecules of the reductive citric acid cycle. The mining of the Beilstein database thus suggests the prebiotic synthesis of the reductive Krebs cycle. We are examining the network catalytic set of molecules to see what is special about the Krebs cycle. In addition, we are now mining the domain of CHNO molecules to examine the expansion of primordial metabolism to compounds that also contain nitrogen. The paper dealing with CHO molecules has been accepted by *PNAS (Proceedings of the National Academy of Sciences of the United States of America)* and will be published soon. This material has been presented at the *Annual Meeting of the Science Advisory Board of the Santa Fe Institute*.

HIGHLIGHTS

- Because internal geophysical processes in a planet generate geochemical energy sources that can support life, the surface conditions of a planet may be irrelevant to whether it hosts life. Therefore, most spectroscopic investigations of distant objects will be inconclusive.

Field Expeditions

Shock's group conducts related fieldwork in hydrothermal ecosystems, which is supported by NSF. Nevertheless, what we learn influences our research done in astrobiology.

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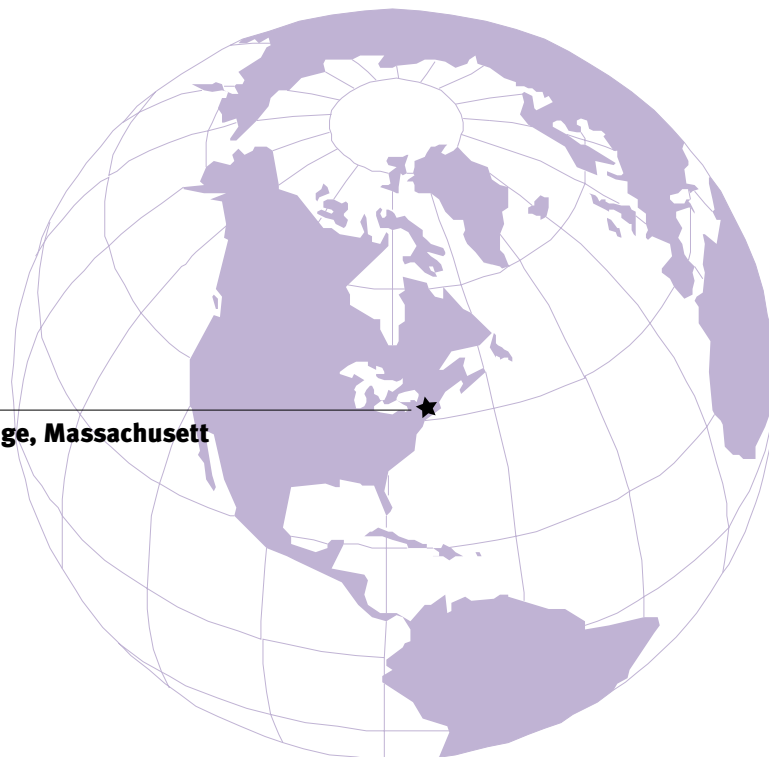
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HARVARD UNIVERSITY

Cambridge, Massachusetts



PRINCIPAL INVESTIGATOR



Andrew H.
Knoll

HAR TEAM MEMBERS

Ariel Anbar,
Harvard University

G. Arnold,
University of Rochester

J. Barling,
University of Rochester

Sam Bowring,
*Massachusetts Institute Of
Technology*

Kevin Boyce,
Harvard University

S. Carti,
University of Rochester

Stephen di
Benedetto,
*Massachusetts Institute Of
Technology*

Douglas Erwin,
Smithsonian Institution

John Grotzinger,
*Massachusetts Institute Of
Technology*

Galen Halvorsen,
Harvard University

John Hayes,
*Woods Hole Oceanographic
Institute*

PROPOSAL EXECUTIVE SUMMARY (1998)

A team of microbiologists, paleontologists, stratigraphers, sedimentary geologists, geochemists, and tectonic geologists has been assembled with the common goal of understanding the coevolution of life and environments in Earth history. Research on the coevolution of Earth and its biota is founded on a careful reading of the supracrustal rock record that accumulated during critical intervals of biospheric change. Interpretation begins with stratigraphy, the careful documentation of time and space as recorded by sedimentary and volcanic rocks. Paleontological, geochronological, biogeochemical, and other geochemical data can be collected simultaneously within this framework, yielding insights into the nature of and relationships between biological and environmental change through time. The formal integration of our research presents an opportunity to establish new standards for investigation of Earth's biological history, an area of fundamental concern to astrobiology. While our principal goal is to elucidate patterns of biological and environmental change on the Earth, the techniques and protocols we establish in the course of this investigation will be equally important in any but the most superficial investigation of other planetary surfaces.

Our proposed research will focus on four critical intervals of Earth history, plus Mars and other planetary exploration technologies:

1. The Archean-Proterozoic Transition (2500-2000 Ma)

This period represents one of the great turning points in Earth history. For the first time, extensive cratonic platforms containing well-developed, deep-to-shallow water sedimentary successions are preserved. These rocks contain a record of global environmental change, including evidence for continental glaciation and an increase in atmospheric oxygen concentrations. The oldest records of eukaryotic cells also occur in rocks of this age.

We propose three specific projects to address key biological and environmental issues of Archean-Proterozoic transition:

- Interdisciplinary research on the Late Archean Campbellrand-Kuruman Platform, South Africa, in which we will:

Year 2

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- a) Establish a high-resolution chronostratigraphic framework using sequence stratigraphic techniques and U-Pb zircon dating of volcanic ash beds.
- b) Document the paleobiological record of latest Archean life within this framework, conducting both micropaleontological research and a focused search for the oldest molecular geochemical record of life on Earth.
- c) Characterize possible fossil structures recently discovered in Campbellrand carbonates, which are identical in size and shape to recently reported "nanobacteria" from Phanerozoic rocks, as well as Mars meteorite ALH84001.
- d) Examine isotopic abundances in carbonates across a paleoceanographic depth gradient of more than 1000m and analyze fluid inclusions preserved in marine cements precipitated as crusts directly on the sea floor, to gain insights into the chemistry of the Archean ocean and atmosphere.

- New chemical techniques for the investigation of Archean and Paleoproterozoic environments. These will include high-precision analyses of redox-sensitive trace metals in latest Archean and Paleoproterozoic black shales, plus measurement of the isotopic composition of Cu, Zn, and Mo, metals expected to be fractionated during uptake by microorganisms. The goal is to provide a detailed record of environmental transition during the Paleoproterozoic "oxygen revolution" that can be integrated with other records of biogeochemical, climatic, tectonic, and biological change.
- New imaging techniques for the investigation of Archean and Paleoproterozoic microfossils. We propose to apply X-ray microscopy and X-ray spectroscopy to study the Earth's earliest microfossils, with the aim of obtaining metabolically informative microchemical data on individual fossils.

2. The Proterozoic-Cambrian Transition (ca. 800-509 Ma)

As in the beginning of the Proterozoic Eon, pronounced climatic, tectonic, biogeochemical, and evolutionary changes are concentrated near the Proterozoic-Cambrian boundary. In these younger, more numerous, and better preserved successions, we plan to test specific hypotheses concerning how different components of the Earth surface system interacted to facilitate the rapid diversification of animals.

Kai-Uwe Hinrichs,
*Woods Hole Oceanographic
Institute*

Paul Hoffman,
Harvard University

Heinrich Holland,
Harvard University

B. Holman,
University of Rochester

K. Knab,
University of Rochester

Andrew Knoll,
Harvard University

Adam Maloof,
Harvard University

Charles Marshall,
Harvard University

Mark Martin,
*Massachusetts Institute Of
Technology*

M. Polizzotto,
University of Rochester

E. Ramon,
University of Rochester

J. Roe,
University of Rochester

Daniel Schrag,
Harvard University

Alex Sessions,

*Woods Hole Oceanographic
Institute*

David Smith,

*Massachusetts Institute Of
Technology*

Roger Summons,

*Australian Geological Survey
Organization*

Sean Sylva,

*Woods Hole Oceanographic
Institute*

Elisabeth Valiulis,

Smithsonian Institution

Wesley Watters,

*Massachusetts Institute Of
Technology*

Shuhai Xiao,

Harvard University

Wenbo Yang,

Harvard University

We propose three principal projects:

- Interdisciplinary stratigraphic, geochronological, and biogeochemical research on thick, well preserved, and exceptionally well exposed successions of Neoproterozoic rocks in Namibia and Svalbard, with the goal of understanding the close stratigraphic relationships among climatic change, tectonism, and secular variation in the Neoproterozoic carbon cycle
- Integrated paleobiological, geochemical, and geochronological investigation of terminal Proterozoic rocks from South China, with the twin goals of adopting new paleontological strategies to fill in the earliest record of animal evolution and understanding how Neoproterozoic environmental change affected the course of early animal evolution.
- Geochronological investigation of massive flood basalts that may have triggered a strong and biologically important perturbation of the marine carbon cycle at the Proterozoic-Cambrian boundary.

3. Permian-Triassic Boundary (251 Ma)

The end-Permian mass extinction eliminated about 90% of all animal species in the oceans. Unlike the well-known Cretaceous-Tertiary mass extinction that eliminated dinosaurs and many other groups, there is no evidence for the impact of an extraterrestrial object at the Permo-Triassic boundary. There is, however, pervasive evidence for extensive environmental perturbation, much of it recorded in shifts in the isotopic records of carbon, oxygen, sulfur and strontium. The temporal precision available in Permian-Triassic successions provides an exceptional opportunity to investigate the relationship between these environmental perturbations and the biotic crisis.

For this study, we plan to:

- Investigate the temporal relationship between well documented and precisely calibrated marine events in southern China and marine and terrestrial perturbations elsewhere.
- Document the distribution of fossil disappearances in the context of geochemical and physical perturbations.
- Examine the sedimentary and biogeochemical consequences of P-T extinction, with the goal of establishing by non-taxonomic means the severity of mass mortality in the end Permian oceans. (In essence, we hope to establish how closely post-extinction sediments match Neoproterozoic sediments deposited before the emergence of animals.)
- Analyze redox-sensitive metal abundances in Permo-Triassic black shales and carbonate precipitates, with the goal of testing oceanographic models for extinction.

4. The Early Archean Eon (ca. 3500 Ma).

Volcanic and sedimentary successions in the Barberton region of southern Africa and the Pilbara Craton of Australia constitute the oldest little metamorphosed supracrustal rocks known on Earth. Despite more than thirty years of study, the biological, tectonic, and environmental interpretation of these rocks remains ambiguous. We propose a new generation of research on the supracrustal succession of the Barberton region that will take full advantage of modern geochemical techniques and phylogenetic inferences. We also plan to examine recently discovered (by team member Bowring) small remnants of supracrustal rocks including iron formation and possible hot spring deposits, that occur as roof pendants in the 4.03 Ga Acasta gneisses, Canada. We estimate that the rocks

are at least 3.6 Ga and may be fundamentally different from the Barberton rocks, in that detrital zircons indicate proximity to much older silicic crust.

5. Mars and Other Planetary Exploration

The paleobiological and paleobiogeochemical exploration of Mars will require remote sensing and sampling technologies not used in terrestrial research. However, the logic and procedures of Martian field work and sampling derive directly from our experience in ancient terrestrial terrains. Also, laboratory analyses of Martian samples will be those successfully employed in the investigation of Earth's biological history. For this reason, an important aspect of the research proposed by our team will be to participate in the development and testing of novel technologies for planetary exploration. We believe that our team has unparalleled experience in field and laboratory studies of ancient terrestrial rocks, and we look forward to using that experience in testing new instruments and approaches developed by members of the NASA Astrobiology Institute.

A critical feature of our proposed research is the search to distinguish general relationships governing major episodes of environmental evolution (likely to hold on many planets), from the individual particularities of life on this planet. By examining these general features in Earth's past, we will provide data and insights of direct relevance to understanding the development of planetary-scale ecosystems.

To complement our research efforts, we propose a program of training and public education. This program will include: (1) undergraduate seminars designed to foster interdisciplinary thinking; (2) regular team seminars for graduate students and postdoctoral fellows, which will (in time) incorporate the Next Generation Internet for weekly meetings of all team members; and (3) public outreach through (a) lecture series at the Smithsonian Institution and Boston Museum of Science, (b) advising on development of a traveling exhibit planned by the Smithsonian, and (c) participation in teacher training workshops organized by the Marine Biological Laboratory.

The several institutions involved in this proposal have made long-term commitments to support of the scientists and laboratories proposed for participation in the NASA Astrobiology Institute. As a lead institution, Harvard will provide administrative and computer support for the team. Team members are committed to the virtual institute concept, in the expectation that it will establish new horizons in research and education.

Roadmap Objectives

#4

Genomic Clues to Evolution

#5

Linking Planetary & Biological Evolution

#6

Microbial Ecology

#8

Past and Present Life on Mars

Project

The Planetary Context of Biological Evolution. Research on Terminal Proterozoic Evolution and the Coevolution of Life and Environments

Senior Project Investigator(s):

Andrew H. Knoll

ACCOMPLISHMENTS

Pursuant to the Harvard NAI team's articulated focus on the coevolution of Earth and life across the Proterozoic-Cambrian transition, we have completed two paleontological studies of early animal evolution. Well-skeletonized animals are generally thought to have arisen as part of the Cambrian "explosion" of animal diversity; however, reefs in the terminal Proterozoic Nama Group (Namibia in southwestern Africa) contain abundant and relatively diverse skeletonized fossils. With NAI team member, John Grotzinger, we have completed computer-assisted, three-dimensional reconstruction of the animals, showing them to be cnidarian-grade invertebrates not closely related to Cambrian shelly fauna. Coeval shales in South China contain another type of fossil assemblage previously unknown from Precambrian successions. Burgess Shale-type compressions from the terminal Proterozoic Doushantuo Formation preserve a remarkable window on biological diversity just before the Cambrian explosion, including diverse algae and two taxa of probable animals. Again, the animals are cnidarian-like and do not extend the record of crown group bilaterian body plans downward into the Proterozoic. In concert, these new windows on early evolution make it clear that whatever the antiquity of the animal kingdom, the characteristic body plans associated with bilaterian phyla arose only in the Cambrian Period.

With Ariel Anbar, we have been working to understand the stratigraphic coincidence between a major carbon cycle shift and the diversification of eukaryotes 1200-1000 million years ago. We propose that both are linked to trace metal abundance in Proterozoic oceans. Only in 1200-1000 Ma, did oxygenation of deep oceans begin to eliminate sulfidic deep water, making Mo bioavailable for the first time (and allowing eukaryotic algae to incorporate nitrate).

With NAI colleagues from the Carnegie Institution, we are using the tools of analytical geochemistry to probe the tissue-level physiology of early land plants. We have examined the evolution of lignin chemistry using ion microprobe, NMR, and soft-X-ray analysis of Early Devonian fossils. Today, vascular plants conduct water in lignin-lined tracheids. Our results show that while stem land plants possessed both complex polyphenolic chemistry and elongated conducting cells, the polyphenolics were localized in surface tissues, only later to become associated with conducting tissues.

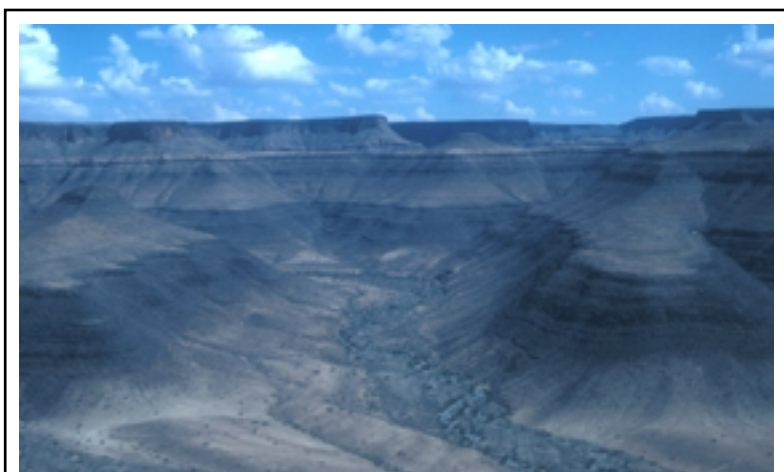
Research accomplished in Year 2 approximated research planned at the onset of the year. In Year 3, we plan to complete further field and laboratory-based studies of evolution and environmental change in both Namibia and China. We also plan to complete the initial phase of collaboration with the Carnegie team and complete geochemical tests of the hypotheses raised in the Anbar/Knoll project.

HIGHLIGHTS

- New and exceptionally well preserved paleontological windows on early biology are providing an unprecedented view of biological diversity before the Cambrian explosion.
- Significantly, these fossils do not extend the record of Cambrian (and modern) animal body plans below the Cambrian boundary, confirming that most animal body plan evolution took place during the Cambrian Period. [See Grotzinger et al. (in press: *Paleobiology*) and Xiao et al. (in press: *Journal of Paleontology*) in the publication list for this report.]

Field Expeditions

Namibian fieldwork was done by Grotzinger in summers of 1998, 1999, and 2000. A.H. Knoll participated in 1998 and 2000, with Steven de Benedetto (MIT graduate student) participating in 1999 and 2000. Purpose of this fieldwork is research on sedimentary architecture and paleontology of a key terminal Proterozoic succession.



An important terminal Proterozoic field site in Namibia.

Cross Team Collaborations

See item in the above report about Carnegie collaboration (using tools of analytical geochemistry to probe the tissue-level physiology of early land plants). Carnegie collaborators include R. Hazen, G. Cody, and M. Fogel. Harvard graduate student, Kevin Boyce, spent a month in residence at Carnegie in May 2000. To date, three abstracts have been published, and we anticipate at least two papers in the next six months.

Roadmap Objectives

- #4 Genomic Clues to Evolution
- #5 Linking Planetary & Biological Evolution
- #6 Microbial Ecology
- #8 Past and Present Life on Mars

Project

The Planetary Context of Biological Evolution. Research on Terminal Proterozoic Evolution and the Coevolution of Life and Environments

Senior Project Investigator(s):
Sam Bowring

ACCOMPLISHMENTS

In the past year, we made considerable progress with three new research projects:

Lower-middle Cambrian study in South China (in conjunction with D. Erwin, A. Knoll).
We visited several new sites in China in January and collected about 12 samples of volcanic rocks possibly interbedded with the spectacular soft-body fossils of the Chengjiang area. This is a critical interval in animal evolution; however, at the present time we do not know the age of these spectacular rocks. They are commonly referred to as 530 Ma, though they may be younger than 520 Ma, which would make a big difference for interpretation of their evolutionary significance. We are currently processing the samples, and more field work is planned.

Evaluating a prediction of the "Snowball Earth" hypothesis.
We have made progress in evaluating a prediction of the "Snowball Earth" hypothesis, which predicts that the global cover of sea-ice should have lasted at least 10 Ma and perhaps longer. In our work on this, we are attempting to constrain the duration of glaciations on the rock record by dating volcanic rocks both above and below the glacial deposits. One of the best places to study this is in Newfoundland. Here, we have identified volcanic rocks below the glacial interval that are younger than 592 Ma and an ash directly overlying Ediacaran fossils that is 575 Ma. It is noteworthy that there are > 5 km of turbidite deposits between the top of the glacials and the 575 Ma ash. Thus, we have constrained the glacial deposits to have been deposited in less than 15 Ma. Further work is ongoing to bracket this interval more tightly.

Constraining the age of the putative oldest trace-fossils from India.
As a part of our work to constrain the age of the putative oldest trace-fossils from India, Mark Martin spent 8 days collecting volcanic and clastic rocks in the Vindhyan Supergroup in central India. Study of these samples supports our work to constrain the age of the Vindhyan supergroup and the age of purported trace fossils found in clastic rocks believed to be approximately 1.0 Ga. Volcanic rocks were only found stratigraphically below purported trace fossils. Preliminary U-Pb zircon analyses indicate that these volcanic rocks are approximately 1.6 Ga. U-Pb detrital zircon studies are in progress from sandstones collected at the same stratigraphic horizon as trace fossils and also from above the trace fossil horizon. This is done in order to place a minimum depositional age on the these sandstones and trace fossils.

HIGHLIGHTS

Field Expeditions

Doug Erwin and Bowring collected from volcanic ashbeds and associated material for

study of the earliest Cambrian sections in Kunming region, Yunnan (southern China) in January 2000.

In February 2000, Bowring and Mark Martin collected volcanic and clastic rocks in the Vindhyan Supergroup in central India.

Bowring traveled to Newfoundland in 1999 to collect ash-beds and examine fossils.

The Planetary Context of Biological Evolution. Research on Terminal Proterozoic Evolution and the Coevolution of Life and Environments

Project

Senior Project Investigator(s):
John P. Grotzinger

ACCOMPLISHMENTS

Sedimentary successions in Namibia (western Africa) and Oman (Arabia) preserve exceptional records of life and environments just before the Cambrian explosion of animal life. Understanding the seminal diversification of animals in the Cambrian requires that antecedent states of life and environment be understood in detail. John Grotzinger has led combined field and laboratory efforts to understand this critical interval, based on Namibian and Omani successions. In Namibia, detailed field research has established the temporal and sequence stratigraphic framework for paleobiological and paleoenvironmental research. In Oman, this has been accomplished by field research, analysis of cores, and seismic data (provided by Petroleum Development Oman). Both regions preserve extensive development of carbonate platforms, and microbial reefs in both regions provided habitats for the earliest known skeleton-forming animals. Careful computer-assisted reconstruction of Namibian fossils reveals a range of cnidarian-grade morphologies, each occupying a particular range of environments in and around the microbial reefs. Omani observations confirm and extend this ecological picture. Further, new geochronological and chemostratigraphic data from Oman provide new insights into the timing and consequences of a major carbon-cycle perturbation at the Precambrian-Cambrian boundary.

In other research, Grotzinger continues to develop a capability for remote field mapping, a capacity that will serve us well in future Mars exploration. He also continues to develop his mathematical model for stromatolite growth, enabling one to quantify the relative effects of biology, physical environment, and chemical conditions on stromatolite growth form. Without such understanding, we cannot hope to gain maximal paleobiological information from these ubiquitous Precambrian sedimentary features. We will also not have confidence in assessing the possible biological role in generating such laminated precipitates as may be found on Mars.

Roadmap Objectives

- #4 Genomic Clues to Evolution
- #5 Linking Planetary & Biological Evolution
- #6 Microbial Ecology
- #8 Past and Present Life on Mars

Roadmap Objectives

#4

Genomic Clues to Evolution

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Linking Planetary & Biological Evolution

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Microbial Ecology

#8

Past and Present Life on Mars

HIGHLIGHTS

Field Expeditions

Namibian fieldwork was done by Grotzinger in the summers of 1998, 1999, and 2000. A.H. Knoll participated in this during 1998 and 2000, and Steven de Benedetto (MIT graduate student) participated in it during 1999 and 2000. The purpose of this fieldwork was research on sedimentary architecture and paleontology of a key terminal Proterozoic succession.

Grotzinger did Oman research in fall 1999 and during late May to June, 2000. This fieldwork purpose was research on sedimentary architecture and paleontology of a key terminal Proterozoic succession.

Cross Team Collaborations

See item in A. Knoll's report above, about our collaboration with Carnegie (using tools of analytical geochemistry to probe the tissue-level physiology of early land plants). Carnegie collaborators include R. Hazen, G. Cody, and M. Fogel. Harvard graduate student Kevin Boyce spent a month in residence at Carnegie in May 2000. To date, three abstracts have been published, and we anticipate at least two papers in the next six months.

Project

The Planetary Context of Biological Evolution. Research on Terminal Proterozoic Evolution and the Coevolution of Life and Environments

Senior Project Investigator(s):
Heinrich Holland

ACCOMPLISHMENTS

Research supported by the NAI grant has dealt largely with trace elements in carbonaceous shales and with a section through the ca. 2.25 Ga Hekpoort paleosol near Garbarone (southern Africa).

We have shown that redox-sensitive trace elements are not enriched in several highly carbonaceous shales that are older than ca. 2.3 Ga. However, they are strongly enriched in the 1.5-1.6 Ga carbonaceous shales we have studied. These results are consistent with the proposed rapid rise of atmospheric oxygen between 2.3 and 2.0 Ga. Under low O₂ conditions, we have found that the redox-sensitive elements U, V, Cr, Mo, and Re are not oxidized during weathering, are not transported in solution to the oceans, and are not concentrated in carbonaceous sediments. In contrast, the ratios of the concentration of redox sensitive trace elements to organic carbon in the 1.5-1.6 Ga shales we have analyzed are virtually identical to the ratios in Devonian black shales. This suggests that the intensity of oxidative weathering during the 1.5-1.6 Ga period was quite similar to that during the Phanerozoic.

The ca. 2.25 Ga Hekpoort paleosol in southern Africa is one of the most extensive and

Year 2

best studied of the Precambrian paleosols. Discovery of what appears to be a nearly complete section through this paleosol near Gabarone has made it possible to define rather precisely the conditions under which it was formed. The presence of a ferricrete horizon at the top of the paleosol proves that the atmosphere contained some O_2 during soil formation. However, the relatively small fraction of FeO in the parent basalt that was oxidized to Fe_2O_3 indicates that O_2 levels were much lower than today, probably between 10^{-4} and 10^{-3} atmospheres. Interpretations of the paleosol, as a ground water laterite formed under high atmospheric O_2 conditions, are difficult to reconcile with the distribution of the major and minor elements in the paleosol. The new paleosol section therefore supports the notion that the Hekpoort paleosol developed during the early stages of the Great Oxidation Event.

Our research this past year has added important new data relating to the evolution of atmospheric O_2 . Results are consistent with the model developed during the past decade, but a good deal more work is needed to define the course and the reasons for the Great Oxidation Event.

The Planetary Context of Biological Evolution. Research on Terminal Proterozoic Evolution and the Coevolution of Life and Environments

Project

Senior Project Investigator(s):
Paul Hoffman

ACCOMPLISHMENTS

Field-based research continues on Neoproterozoic ice ages and their effects on biological evolution. The Snowball Earth hypothesis, recently rearticulated by Hoffman and Schrag, posits that the Earth was plunged into long periods of global glaciation repeatedly between 800 and 575 million years ago. Unusual geological and geochemical features found in association with glaciogenic rocks can be explained by ice that not only reached sea level at the equator, but spread across the oceans to create a "snowball Earth." How life survived and responded to these perturbations is of great interest to anyone interested in the long term persistence of life on a planet.

Detailed stratigraphic studies are currently being conducted in Namibia (southwestern Africa) and Spitsbergen (Arctic Norwegian island group), with samples for geochemical analysis collected in a framework of stratigraphic sequences. New data from the current year's research include geochemical and sedimentological evidence for pre-Varanger glaciation in Spitsbergen, as well as periglacial features of a type predicted by the snowball model in Mauritania (northwestern Africa).

HIGHLIGHTS

Field Expeditions

During summers of 1999 and 2000, fieldwork was done on the Otavi Group (northern

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Project

The Planetary Context of Biological Evolution. Research on Terminal Proterozoic Evolution and the Coevolution of Life and Environments

Senior Project Investigator(s):
John Hayes

ACCOMPLISHMENTS

Work at Woods Hole has focused on methane biogeochemistry and on hydrogen-isotopic biogeochemistry. Available funds supported a six-month visit by Dr. Roger E. Summons, Chief Research Scientist in the Australian Geological Survey Organisation and a Co-Investigator of the Harvard astrobiology group. These funds also contributed to salary and research expenses for people affiliated with the Woods Hole Oceanographic Institution (WHOI): (1) Sean P. Sylva, Research Associate; (2) Alex L. Sessions, doctoral student working on hydrogen-isotopic studies; and (3) Dr. Kai-Uwe Hinrichs, post-doctoral scholar working on methane biogeochemistry.

Hinrichs, with assistance from Sylva, has continued to study molecular biomarkers from sediments in which methane is being oxidized anaerobically. Our first publication in the present series (Hinrichs et al., 1999) reported the discovery of archaeal lipids that were strongly depleted in ^{13}C and which, therefore, apparently derived from methanotrophs rather than methanogens. A 16S rRNA gene library from the same sediments (Eel River Basin, offshore northern California) yielded numerous phylotypes that were related to but distinct from known methanogens. The most prominent group branched deeply enough that it could represent a new order within the methane-related archaea. Accordingly, we proposed that the ^{13}C -depleted biomarkers were products of obligately methanotrophic archaea and that the archaeal domain contains organisms capable of consuming methane as well as producing it.

Earlier studies of the anaerobic oxidation of methane have shown that the overall process involves transfer of electrons from methane to sulfate. A microbial consortium involving both methane-consumers (now shown to be archaeal) and sulfate-reducers has, therefore, been postulated. In work during the present year, Hinrichs, Summons, and Sylva have extended our earlier investigations and shown that a large group of ^{13}C -depleted biomarkers co-occurring with archaeal products must be of bacterial (= eubac-

terial) origin. The definitive molecular structures within this group are n-alkyl and ether-linked (Hinrichs et al., 1999, 2000). Glycerol ethers are extremely rare among bacterial products, but they are not unique. They have been reported previously in thermophilic sulfate-reducing bacteria. We believe, therefore, that we have now identified products from organisms of both types within the hypothesized consortia: ^{13}C -depleted isoprenoidal glycerol ethers from the archaea and ^{13}C -depleted n-alkyl glycerol ethers from the bacteria. The efficient transfer of methane-derived (isotopically depleted) carbon from the methane consumer to the sulfate reducer indicates that CO_2 is not the main product of methane consumers. Our evidence is being utilized productively by microbial ecologists dealing with that issue.

Follow-up studies are in progress. On the archaeal side, we have found that the molecular structures deduced and described in our earlier report (Hinrichs et al., 1999) were correct. However, the chemical derivatives formed in the course of our analyses, and for which the mass spectra are shown, actually contain only one trimethylsilyl ether moiety rather than two. To prevent confusion among subsequent investigators, we have published a note clarifying this point (Hinrichs et al., 2000). On the bacterial side, further work is showing that n-alkyl glycerol ethers are widely distributed in anaerobic sediments. This apparently indicates that the sulfate reducer involved in the methane-consuming consortium, or its relatives, is a previously unrecognized but important member of sedimentary microbial communities. Hinrichs and Summons are drafting a further report for submission to *Proceedings of the National Academy of Sciences of the United States of America*.

The process in which methane is oxidized anaerobically is notable for its low yield of energy. In separate work, Hayes and coworkers (Hayes et al., 1999) were able to calculate in situ yields of energy rather precisely for the process in sediments from the Kattegat (waterway between Denmark and Sweden). They showed that the yield was barely adequate to sustain two trophic levels and suggested that the example was pertinent to considerations of chemotrophic systems on the early Earth, in the deep bacterial biosphere, and on other planets. A detailed version of this report is in preparation for *G3 (Geochemistry, Geophysics, Geosystems)*, the on-line journal now published by the American Geophysical Union.

Our work on methane oxidation processes was initially motivated by hypotheses that the carbon-isotopic anomaly recorded in marine carbonates of late Paleocene age (55 Ma) reflected a globally significant release of methane from sub-seafloor hydrates. Seeking to confirm this hypothesis, we searched related sediments for any ^{13}C -depleted biomarkers deriving from the methanotrophic bacteria that must have flourished if such releases had occurred. Our results have been consistently negative, even as further evidence (e.g., results indicating that the isotopic anomaly reaches its maximum strength in only two to four thousand years) points increasingly to sudden decomposition of hydrates. One report of these negative findings is now in press (Bolle et al., 2000).

At present, Hinrichs is preparing a review of methane cycling for presentation at the Gordon Research Conference on the Origin of Life. He will, in particular, consider the possibility that the dramatic carbon-isotopic-depletion signal in the late Archean derives from anaerobic, rather than aerobic, processes and will provide estimated mass and

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redox balances for known sedimentary basins.

HIGHLIGHTS

Cross Team Collaboration

Our work on hydrogen-isotopic biogeochemistry provides one example of collaboration with another group within the NAI. Sessions et al. (1999) examined isotopic fractionations associated with lipid biosynthesis and provided preliminary interpretations in terms of hydrogen budgets and isotope effects associated with formation of NADPH (nicotinamide adenine dinucleotide phosphate, reduced form). To examine related phenomena in anaerobic microorganisms, we obtained biomass from six different culture of sulfate-reducing bacteria grown by the Ames astrobiology group. A preliminary report of this work was provided at the Fall, 1999 meeting of the American Geophysical Union (Sessions et al., 1999) and an updated version was presented at the Astrobiology Science Conference (Sessions et al., 2000). In addition, two detailed manuscripts describing techniques and calculations required for high-precision, compound-specific hydrogen-isotopic analyses have been submitted for publication (Sessions et al., 2000).

Further work with the Ames group is underway. In particular, L. Jahnke is growing aerobic, methanotrophic bacteria in media for which we have already characterized all sources of H. Analyses of the products will provide the most complete isotopic and elemental mass balance for H available to date for any chemotrophic organism.

Adding another dimension to the collaboration, Hinrichs and Summons took the opportunity to search the sulfate-reducing-bacterial products just described for n-alkyl glycerol ethers. None were found, confirming that the products are restricted to the deeply branching group that was not represented among the organisms grown at Ames.

A third collaboration has been proposed. We await word on the outcome of our request for an augmentation that would support a series of collaborative investigations with Dr. Mitchell Sogin's astrobiology group at the Marine Biological Laboratory. In that project, we would develop techniques for the isolation of 16S rRNA (the native molecule, not the amplified gene) from natural samples in quantities adequate for isotopic analysis (~ 500 pmol C). The results would provide the first direct evidence capable of linking lipid biomarkers to specific phylotypes. Such a finding would associate metabolic culture processes and pathways of carbon flow with specific organisms without obtaining each species in pure culture.

Project

The Planetary Context of Biological Evolution. Research on Terminal Proterozoic Evolution and the Coevolution of Life and Environments

Senior Project Investigator(s):
Douglas H. Erwin

ACCOMPLISHMENTS

Activities by Co-I Douglas H. Erwin have focused on the following areas:

1. Environmental and biological context of the Cambrian metazoan radiation.

Efforts in this area over the past year have involved various activities: (a) consideration of the biological effects of the proposed Neoproterozoic "Snowball Earth"; (b) collaborative work with Sam Bowring (MIT) on the rate and timing of the evolutionary events; (c) search for new Ediacaran fossils in sub-Nama group sediments in southern Namibia; and (d) continuing effort to use molecular and developmental data to understand the nature of the protostome/deuterostome (PD) ancestor. Study of the PD ancestor is focused on consideration of highly conserved developmental control genes between protostomes (largely *Drosophila*) and vertebrates. Sequence conservation is a poor guide to functional conservation, making it difficult to infer the nature of the PD ancestor unequivocally.

2. Patterns and processes of post-extinction recovery.

Focus has been on the nature of biotic recovery after mass extinctions. I have begun developing models of these processes with colleagues at the Santa Fe Institute.

3. Causes of the end-Permian mass extinction.

Continuing effort to understand the rate and likely causes of the greatest mass extinction in the past 540 million years.

Work for the coming year includes: (1) additional fieldwork in Namibia with colleagues from Harvard and MIT; (2) a continuation and expansion of the modeling effort with colleagues at the Santa Fe Institute; and (3) continued study of comparative developmental biology of the protostome/deuterostome ancestor.

HIGHLIGHTSField Expeditions

Fieldwork for Year 2 described below involved Douglas H. Erwin.

In June 1999, Erwin joined a field trip organized by Paul Hoffman (Harvard) to examine evidence in northern Namibia for late neoproterozoic "Snowball Earth."

In southern Namibia during June 1999, our group examined soft-bodied Ediacaran fossils from sub-Nama Group sequences. Erwin was in this group with Sam Bowring (MIT) and Charlie Hoffman (Namibian Geological Survey).

During July 1999 in northern Russia, our group studied soft-bodied Ediacaran fossils from the White Sea sections north of Archangelsk, Russia. Erwin did this fieldwork with Misha Fedonkin and Dimitri Grahzdankin (Paleontological Research Institute, Moscow) and also with Dolf Seilacher (Yale and Universitat Tubingen).

In Yunnan Province (southern China) during January 2000, we collected material from volcanic ashbeds and their associated material for study of earliest Cambrian sections in the Kunming region, Yunnan. Erwin worked with Sam Bowring (MIT) and Wang Wei (Nanjing Institute of Geology and Palaeontology).

Roadmap Objectives

#4

Genomic Clues to Evolution

#5

Linking Planetary & Biological Evolution

Project

Molecular Paleontology and the Stratigraphic Record

Senior Project Investigator(s):
Charles Marshall

ACCOMPLISHMENTS

Molecular clocks have become a powerful way of calibrating major evolutionary events with the geological record, thus providing the temporal context needed for establishing links between bursts of evolutionary innovation, along with both geological and environmental events. This is especially important in the Precambrian given the dearth of fossiliferous rocks.

However, when molecular clocks have been used to estimate divergence times for groups that have good fossil records, the estimates are typically much older than a literal reading of the fossil record would suggest. It is currently unclear which type of data is giving the better answer.

I continue to work on and develop (with important collaborations) new methods for quantifying the incompleteness of the fossil record in the attempt to reconcile the different temporal frameworks that fossil and molecular data currently yield. The long term goal is to provide a rigorous enough temporal framework, using both molecular and fossil data, for understanding how planetary evolution affects or controls biological evolution.

Roadmap Objectives

#5

Linking Planetary & Biological Evolution

#12

Effects of Climate & Geology on Habitability

#14

Ecosystem Response to Rapid Environmental Change

Project

Redox-Sensitive Metals and Environmental Evolution

Senior Project Investigator(s):
A. D. Anbar

ACCOMPLISHMENTS

There are three primary components to the ongoing research at the University of Rochester in collaboration with the Harvard Team:

- 1) Conceptual study of the impact of the changing Precambrian redox environment on the availability of key nutrient metals (especially Fe and Mo).
- 2) Measurement of concentrations of redox-sensitive metals in Proterozoic sediments to place constraints on atmosphere/ocean redox evolution.

3) Exploration of the use of metal isotope fractionation (esp. Fe and Mo) to study paleoredox conditions and changes in the biological use of metals through time.

Component (1) has developed into a draft manuscript coauthored by Anbar and Knoll. This manuscript discusses the likely impact on Fe and Mo abundances of a sulfidic Mesoproterozoic ocean, as postulated by D. Canfield. Because of the extreme redox sensitivity of Fe and Mo, these metals would have been scarce in seawater under such conditions, with profound consequences for the biological nitrogen cycle and eukaryote evolution. These concepts were presented at the Fall 1999 American Geophysical Union Conference. This component is ahead of schedule, as it was not among the proposed goals. Published results are anticipated in Year 3.

Component (2) has involved efforts by Anbar, Arnold, Ramon, and Barling to develop and apply methods to measure Mo, Re, U, V, and other trace metals in Proterozoic carbonaceous shales. These methods involve sample digestion and analysis using an inductively coupled plasma mass spectrometer (ICP-MS) at the University of Rochester. This work is being coordinated with the similar efforts of H.D. Holland (also a Harvard Team member), who is focusing on Archean shales. Both projects are aimed at looking for evidence of changes in the geochemical cycling of redox-sensitive metals, as a means of constraining the redox evolution of the environment. At Rochester, methods have been finalized in the past 12 months, and work has begun on Mesoproterozoic sediments obtained by A. Knoll from the McArthur Basin, Australia. This component is slightly behind schedule because of the delayed start in funding, but it should be on schedule in Year 3, with published results anticipated in Year 4.

Component (3) is a completely novel application involving extensive development of new analytical methods used for determination of the mechanisms by which metal isotopes are fractionated in nature. This work involved Anbar, Barling, Roe, Knab, Polizzotto, Carti, and Neelson. In Year 2, we developed methods for Fe and Mo isotopic analysis and observed variations in natural materials. Mo variations suggest a possible application for paleoredox studies, which will be a major area of emphasis in Year 3. In work sponsored primarily by the JPL/Caltech Team, we demonstrated that nonbiological chemical processes can fractionate Fe isotopes. This has important implications for biomarker applications. These projects led to presentations at both the 1999 AGU (American Geophysical Union) and GSA (Geological Society of America) Conferences, the 1999 V.M. Goldschmidt Conference, and a publication in *Science* in April, 2000.

In addition during this period, we submitted a manuscript for publication to *Journal of Geophysical Research- Planets* on the influence of impacts on the Hadean (> 3.85 Ga) surface environment. This manuscript is an outgrowth of Ir and Pt measurements in Akilia Island metasediments conducted by Arnold in Year 1. Coauthors on this publication are S. Mojzsis (UCLA Team) and K. Zahnle (NASA Ames Team). Our interpretation of the data leads to the conclusion that the Earth's surface was habitable during most of the "Late Heavy Bombardment."

HIGHLIGHTS

- First demonstration of natural variations in the isotopic composition of molybdenum
- First demonstration that nonbiological chemical processes can fractionate iron iso-

topes (with JPL/Caltech Team)

- Contrary to conventional wisdom, the Earth's surface was habitable during most of the period 4.1 – 3.8 Ga despite frequent large impacts.
- Scarcity of critical nutrient metals in the postulated sulfidic Proterozoic oceans may explain delayed rise of eukaryotes to ecological prominence.

Cross Team Collaboration

1. Collaboration with K. Nealson of the JPL/Caltech Team lead to experiments on Fe isotope fractionation and culminated in a publication in *Science*.

2. Collaboration with S. Mojzsis (UCLA Team) and K. Zahnle (NASA Ames Team) was instrumental in obtaining and interpreting data from Akilia Island metasediments. This has culminated in a publication submitted to *Journal of Geophysical Research-Planets*.

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Year 2

JET PROPULSION LABORATORY



PRINCIPAL INVESTIGATOR



**Kenneth H.
Nealsen**

JPL TEAM MEMBERS

Thomas Ahrens,
California Institute Of Technology

Mark Allen,
California Institute Of Technology

Ariel Anbar,
Harvard University

Daniel Austin,
California Institute Of Technology

Jill Banfield,
University Of Wisconsin Madison

William Barker,
University Of Wisconsin Madison

J. Barling,
University of Rochester

Brian Beard,
University Of Wisconsin Madison

Jesse Beauchamp,
California Institute Of Technology

Geoffrey Blake,
California Institute Of Technology

Diana Blaney,
Jet Propulsion Laboratory

PROPOSAL EXECUTIVE SUMMARY (1998)

We have developed a Consortium comprising four institutions and additional distinguished investigators, led by the Jet Propulsion Laboratory, which we propose as a member of the NASA Astrobiology Institute. The goals of the Consortium are to promote understanding in three related areas central to the mission of the NAI and the search for life in the universe:

- understand the environmental contexts conducive to the maintenance of life on Earth or extraterrestrial planets
- determine the existence and nature of life environments outside the Earth
- identify approaches to confirm the existence of life, extant or extinct, in these contexts

The research plan of the Consortium is built on corresponding themes:
The study of Earth as a laboratory for understanding life and its relationship to its host planet: Given that Earth is where we know that life exists, we will use the Earth and its biosphere as the laboratory for understanding the contexts for life.

The study of Mars as an analog: Mars is the best Earth-like site where we can hope to understand extraterrestrial contexts, how planets evolve, and what possible effects resident biota might have on the evolution.

The identification of biosignatures that can be used to detect and identify life:
We presently lack a suitable array of unambiguous indicators of life (biosignatures) that can be used to interrogate environments or samples where life is suspected to reside. This is evidenced by the recent report of presumptive evidence for Martian life in the meteorite ALH84001.

The objectives of the Consortium are centered on these themes and are achieved through the organized program of research. Individual research tasks are performed by world-class experts who are laboratory and field scientists, modelers and experimentalists. These tasks are carried out within Task Groups that derive from the themes as shown, each task providing an essential element contributing to the goal of that theme.

Year 2

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Earth as a Laboratory: Work on Early Earth Environments will examine different aspects of early Earth evolution—atmospheric chemistry, impact effects, and volcanism—to provide the contextual bases for life. The record of early metabolic innovations (Evolution of Metabolism) will be deduced using genetic records contained in the genomes of extant organisms to extrapolate backward in time. From this work, we will reassemble the early history of metabolism so that it can be compared to the environmental history of the early Earth. Did geology and geological changes drive the evolution of life, or did life drive key changes in the atmosphere and hydrosphere, and thus drive the evolution of the Earth?

Mars as an Analog: The early Earth group will also work closely with the group studying the Early Mars Environment, which will be asking similar questions about Mars. In Mars may lie many of the answers about early Earth. Are differences in their histories due to the effects of the biosphere on planetary evolution—or the lack of them? The Modern Mars Environment group will be concerned with the search for modern environments in which life might have existed or might still exist.

Signatures of Early Life: In essential support of both of these efforts is a major effort on the Development of Biosignatures. Some of these are familiar biosignatures in which we seek new levels of resolution or understanding (e.g., organic carbon signatures; structural signatures as determined by microscopic methods; and isotopic fractionation of C, S, and N). Others represent new types of signatures (e.g., isotopic fractionation of Cu, Zn, and Fe; layered communities; and formation and dissolution of minerals as signatures). These signatures will be used to develop methods for their determination with Earth samples (Biosignatures in the Geological Record). These methods will have wide application to study of the Earth itself, both ancient and modern, as well as the study of extraterrestrial environments and samples returned to Earth. In several instances both modern and ancient Earth samples will be used for testing the methods.

These objectives are interdisciplinary, and many co-investigators are involved with more than one task group. The overall effort is highly innovative, bringing together geobiologists, molecular biologists, microbiologists, and planetary scientists to work together in ways that are entirely new. Out of this will certainly come new directions in astrobiology and, we hope, a foundation for a new generation of exploration in the field. Our Management Plan ensures that the work remains focused on the Consortium goals.

D. Breen,

California Institute Of Technology

Karen Brinton,

California Institute Of Technology

Jeffrey Brownson,

University Of Wisconsin Madison

S. Carti,

University of Rochester

F. Corsetti,

University Of California Santa Barbara

Evan Dorn,

California Institute Of Technology

Marilyn Fogel,

Carnegie Institution Of Washington

W. Fritz

Lisa Gaddis,

USGS Flagstaff

Eric Gaidos,

California Institute Of Technology

James Hagadorn,

California Institute Of Technology

Ken Herkenhoff,

Jet Propulsion Laboratory

J. Hollingsworth,

Union Carbide

B. Holman,

University of Rochester

Clark Johnson,

University Of Wisconsin Madison

Jeff Johnson

Joseph Kirschvink,

California Institute Of Technology

K. Knab,

University of Rochester

James Lyons,

University Of California Los Angeles

Francis Macdonald,

California Institute Of Technology

R. Amaya Martinez

UNAM

Matthew McCarthy,

Carnegie Institution Of Washington

Gene McDonald,

Jet Propulsion Laboratory

Brian Meehan,

California Institute Of Technology

Kenneth Nealson,

Jet Propulsion Laboratory

J. Nesson,

National Institutes of Health

Maria Nesterova,

University Of Wisconsin Madison

Julie O Leary,

University Of Wisconsin Madison

Jeffrey Plescia,

Jet Propulsion Laboratory

M. Polizzotto,

University of Rochester

Tim Raub,

California Institute Of Technology

J. Roe,

University of Rochester

Principal Investigator Kenneth Nealson is responsible for the overall research program, ensuring that the scientific objectives are achieved and that the Consortium is a fully contributing member of the NAI. Dr. Nealson is ideally suited to lead this program with his extensive experience as a leader in interdisciplinary research efforts, training in biochemistry and microbiology, experience in biogeochemistry and molecular biology, and record of service for NASA and the National Academy of Sciences.

Dr. Nealson will be assisted by a Science Steering Group comprising the leaders of the six Task Groups, who together will ensure that the tasks remain coordinated and focused. The leaders of each of the Task Groups are experts in their respective areas with experience in multidisciplinary efforts.

Dr. Nealson chairs a Consortium Board containing high-level representatives from each of the four institutions, who will ensure that all institutional commitments are maintained. Each institution is strongly motivated to see that the Consortium and the NAI succeed, and each has made available significant resources to this end. JPL is committed to the exploration of Mars and the development of a substantial astrobiology program as part of an overall thrust in 'origins' research. They have provided substantial salary support to Nealson and his staff for work proposed here and have provided nearly all of the equipment required at no cost to this proposal. Caltech has established a new program in evolutionary geobiology that espouses many of the same goals as our Consortium. Caltech is also actively recruiting for three new faculty positions in this field. USGS (along with JPL) is fully committed to the exploration of Mars and understanding its history. USGS is eager to add this microbiological dimension to their geology programs. The University of Wisconsin has instituted a geomicrobiology program with strong emphasis on molecular biological approaches. They make state-of-the-art facilities available, critical for the biosignatures work proposed here.

Answers to basic questions posed in the study of early and modern Earth and Mars, as well as the tools (biosignatures) being developed for these studies, will be of great relevance to other NASA programs. First with regard to upcoming missions, the work will be related directly to issues like site selection, sample selection, planning of methods for sample return, sample protection from contamination, and sample analysis. The methods will also have relevance to planning both remote and *in situ* studies of Mars and other solar system objects (including remote studies of Earth), where specific signatures may be of great use in asking questions about our own planet. Finally, understanding the history of the atmospheric chemistry of early Earth will be of great relevance in planning extrasolar missions. If the primitive atmosphere of Earth is known, then similar atmospheres may be sought as evidence of young planets, similar to Earth in its very much alive, but non-oxygen rich, state.

Training is an important part of the effort. The Consortium has training in place for undergraduate and graduate students and postdoctoral fellows through the current education programs of its members. These programs will be extended to include specific training in the disciplines supporting astrobiology, and in methods for multidisciplinary research. The programs envisioned include undergraduate training through mechanisms such as the Summer Undergraduate Research Fellowship (SURF) and Minority Undergraduate Research Fellowship (MURF) Programs; an undergraduate course to be

Year 2

offered through the NGI; and the NASA Planetary Biology Internship (PBI) program. At the graduate level, we will capitalize on our mixture of academic and research-oriented institutions to offer on-the-job rotations for graduate students. We will also offer a graduate course through the Consortium and participate in ongoing graduate education programs such as the NASA-sponsored Graduate Student Researchers Program (GSRP). At the postdoctoral level (in addition to the substantial postdoctoral support requested as part of our proposal), we have a number of training programs in place. These include the National Research Council Resident Research Associateship program that supports postdoctoral and senior researchers to come to JPL, and the NAI postdoctoral program in which we will participate. Finally, we have in place at JPL the NASA/ASEE, which offers summer faculty fellowships (SFF) for faculty from Historically Black Colleges and Universities.

The Consortium is eager to make its contribution to education at K-12 levels and to public understanding in astrobiology. The Consortium will make its work available to the classroom and the public at several levels, and a plan is presented that will accomplish this. It includes working with TERC (an NSF-funded organization) for curriculum development in a national astrobiology program; working with the California State University system for the teaching of high school teachers; and the establishment of a user-friendly astrobiology web page for dissemination of information to the public.

Impact Frustration and Subsequent Generation of Biologically Tenable Climates on Earth and Mars

Project

Senior Project Investigator(s):
Thomas J. Ahrens

ACCOMPLISHMENTS

We are developing a system to measure positive or negative ions. The speciation of gases produced by impact de-volatilization of target rocks in large impact events can have significant environmental implications that affect life on planets such as the Earth and Mars. The presence of anhydrite in the sediments at Chicxulub means that SO_2 and SO_3 were released in large quantities during the K/T impact, resulting in the formation of a stratospheric sulfuric acid layer sufficiently opaque to cool the Earth and interrupt photosynthesis. Atmospheric SO_3 is very rapidly converted to sulfuric acid (H_2SO_4), whereas SO_2 is converted to sulfuric acid on longer time scales (\sim years). This extends the environmental influence of the de-volatilized anhydrite to possibly a ~ 10 year time-scale of drastic global climatic change. Thus, the ratio of SO_2 to SO_3 is a key factor in the extent of cooling following the K/T impact.

We are also currently constructing experiments to employ a pulsed electron gun to ionize virtually all neutral gas species produced by a given shock pressure on a mineral sample. This in-progress work will likely yield a more complete and quantitative mass spectrum of volatilized species. Systematic study of the speciation of impact-induced

James Scott,

Carnegie Institution Of Washington

Joseph Skulan,

University Of Wisconsin Madison

Lawrence Soderblom,

USGS Flagstaff

J. Stewart

USGS

Michael Storrie-Lombardi,

Jet Propulsion Laboratory

Henry Sun,

Jet Propulsion Laboratory

Kenneth Tanaka,

USGS Flagstaff

B. Waggoner

Benjamin Weiss,

California Institute Of Technology

Susan Welch,

University Of Wisconsin Madison

B. Wernicke,

California Institute Of Technology

Susan Ziegler,

Carnegie Institution Of Washington

Roadmap Objectives

#14

Ecosystem Response to Rapid Environmental Change

vapor produced from impact of biogenetic related CO_2 -, SO_2 -, and H_2O -bearing minerals is being conducted.

In order to address the issue of sulfur speciation in an impact, we are developing mass spectrometry methods associated with performing laboratory impact experiments on natural anhydrite samples. These methods use Dr. Hörz's 4 ns pulsed N_2 laser (337 nm) as the energy source for UV-visible emission and time-of-flight mass spectrometry (TOFMS) experiments. The UV-visible emission experiments were performed in air using a system built for study of the calibration of the Cosmic Dust Analysis instrument. Thus far, these experiments have yielded spectra dominated by Ca and CaO emissions, but without lines from any sulfur species. The lack of detectable sulfur species may be due to the relatively weak visible emission intensities of these species compared to Ca species. Time-of-Flight Mass Spectrometer (TOFMS) experiments were carried on in vacuum at 10-15 on an instrument in Dr. Beauchamp's laboratory with a one meter path length and a 15 kV target plate voltage. In addition to the low ionization potential metals (Na+, K+, Ca+, Mg+, etc.), several sulfur species were observed, including SO_4^+ , CaS^+ , and CaSO_2^+ . Notably, ions of SO_3 were not observed in these experiments. Therefore, the results obtained do quantitatively constrain the SO_2/SO_3 ratio to ~ 10 .

HIGHLIGHTS

- Developing Modes to Study Sulfur Speciation in an Impact
 - Mass spectrometry methods for laboratory impact experiments on natural anhydrite samples
 - UV-visible emission and time-of-flight mass spectrometry (TOFMS)
- Findings Thus Far
 - Spectra dominated by Ca and CaO emissions without lines from any sulfur species (Lack of detectable sulfur species may be due to relatively weak visible emission intensities of these species compared to Ca species.)
 - In addition to the low ionization potential metals (Na+, K+, Ca+, Mg+, etc.), several sulfur species were observed, including SO_4^+ , CaS^+ , and CaSO_2^+ .
 - Notably, ions of SO_3 were not observed in these experiments. Therefore, the results obtained do quantitatively constrain the SO_2/SO_3 ratio to ~ 10 .

Field Expeditions

Visit to NASA/JSC : Experimental tests using their pulsed electron gun to support our research endeavors. We are currently constructing experiments to employ a pulsed electron gun to ionize virtually all neutral gas species produced by a given shock pressure on a mineral sample.

Year 2

Mars Atmospheric Model

Project

Senior Project Investigator(s):
Mark Allen

ACCOMPLISHMENTS

We modernized our Mars atmospheric model to allow introduction of "exotic" species, i.e., potential gaseous biomarkers. This was done to be able to estimate atmospheric life times and dispersal potentialities. This work is still in progress.

Roadmap Objectives

#8

Past and Present Life
on Mars

Fractionation of Transition Metal Isotopes

Project

Senior Project Investigator(s):
A. D. Anbar

ACCOMPLISHMENTS

The primary focus of this research is to determine if fractionations of transition metal isotopes can be used as biomarkers. We are also interested in the possible use of such fractionations to study changes in metal geochemical cycling through time. This could provide insight into past changes in environmental conditions and/or changing use of metals in biochemistry. Biomarker applications are of primary interest to the JPL/Caltech Team. The other applications are also of interest to the Harvard Team, which also supports this research.

In Year 1 and 2, most of our focus was on methods development and initial applications, with iron and molybdenum isotopes getting the bulk of attention. The most relevant finding in biomarker studies was our demonstration that Fe isotopes can be fractionated by chemical processes in the absence of biology. This was demonstrated using ion exchange chromatography. Our interpretation of the data is that fractionation occurs during equilibration between dissolved Fe complexes in solution, which suggests that some of the natural variations in Fe isotopic composition may be due to non-biological chemistry. This interpretation also provides insight into the mechanism(s) of biological Fe isotope fractionation. This work was presented in 1999 at these meetings: *V.M. Goldschmidt Conference*, *GSA (Geological Society of America)* and *AGU (American Geophysical Union)*. It was published in *Science* in April, 2000.

We intend to follow up on these findings in Year 3 by: (1) conducting non-biological experiments using systems more relevant to nature than those used to date; and (2) exploring natural systems where biological influence is unlikely.

We also observed natural variations in the isotopic composition of Mo, which suggest

Roadmap Objectives

#5

Linking Planetary &
Biological Evolution

#7

Extremes of Life

#8

Past and Present Life
on Mars

Roadmap Objectives

- #1 Sources of Organics on Earth
- #5 Linking Planetary & Biological Evolution
- #6 Microbial Ecology
- #8 Past and Present Life on Mars
- #12 Effects of Climate & Geology on Habitability
- #13 Extrasolar Biomarkers

a possible application for paleoredox studies. This will be a major area of emphasis in Year 3 in collaboration with the Harvard Team. Two manuscripts on Mo isotopes are in preparation.

HIGHLIGHTS

- First demonstration that nonbiological chemical processes can fractionate Fe isotopes (with Harvard Team)
- First demonstration of natural variation in Mo isotopic composition (with Harvard Team)

Cross Team Collaborations

We have collaborated with H.D. Holland and A.H. Knoll of the Harvard Team to develop a conceptual framework for the study of Mo isotopes in Precambrian sediments. Details of this collaboration are found in the Harvard Team progress report.

Project

Biological Influences on Weathering

Senior Project Investigator(s):

Jill Banfield, William Barker

ACCOMPLISHMENTS

Biological activity can generate minerals with unique compositions, structures, and forms that may persist long after organic remains of cells associated with biomineralization are destroyed. Thus, laboratory studies of mineralization on, or in proximity to, organic polymer assemblages are of direct relevance to interpretation of biosignatures. We are exploring the linkages between structure and properties of organic polymers (such as those associated with cell surfaces), solution chemistry, and the resulting mineral-polymer composites. Biomimetic experiments are being conducted on iron oxyhydroxide-polymer systems where the iron oxyhydroxide is ferrihydrite or goethite (FeOOH). The work has required development of a number of new biomimetic composite materials that have been synthesized through such strategies as carbohydrate self-assembly, multiphase amphiphilic self-organization, and "guest-host" inorganic phase formation. Two- and three-dimensional matrices (templates based on natural functional polysaccharides) were observed to host the formation of iron (III)-containing oxo-hydroxyphases of various morphologies. Close connection between polymer matrices and mesoscopic mineral phase morphologies was revealed in some cases. This work is primarily being conducted by Dr. Maria Nesterova, who joined our group shortly after the NASA funding became available in November 1999. She will present an invited talk at an AGU (*American Geophysical Union*) Meeting, reporting results of the first 5 months of her work.

In parallel with the above mentioned laboratory studies, high-resolution and analytical transmission electron microscopy are being used to study biomineralized sheaths and

stalks of a variety of iron-oxidizing microorganisms that form in a subsurface aquifer. All sheaths were heavily mineralized by ferric iron minerals, especially ferrihydrite and ferroxihite. Bacterially-mediated ferrous iron oxidation is followed by immediate precipitation due to the extremely low solubility of ferric iron at pH ~8.0. The resulting particle size is extremely small, typically 2-3 nm. Particles coat all polymer surfaces, leading to distinctive polymer-mineral assemblages, which form colloidal aggregates due to flocculation of nanoparticles in solution.

In addition to understanding the ways in which biomineralization occurs, it is important to understand how subsequent reactions (due to heat and / or time) can modify mineralogical biosignatures. This is especially important when particle size is very small, because the large driving force associated with surface free energy promotes rapid crystal growth. Characterization of the mineralized sheaths and stalks, as well as the colloidal aggregates of nanoparticles, demonstrated that crystal growth occurs primarily by a novel mechanism that involves oriented attachment of very small particles. This is most pronounced at the margins of polymers, where rotation of particles is not restricted by binding to the polymeric substrate. These findings lead to the prediction that the preexistence of cell and distinctive cell product morphologies might be detected using analysis of both particle size distribution patterns and defect microstructures. (The organic substrate should be evident due to the finer particle size). This research has been submitted for publication and will be published following some revision (Banfield et al. *Science*, in press).

In related work, Mr. Jeffrey Brownson (an MS student) joined our group in Fall 1999. He is working on experimental biomineralization of clay minerals on cell surfaces.

HIGHLIGHTS

- Our paper on crystal growth in biomineralization products has just been accepted for publication in *Science*. This work received partial support from NASA. The abstract of this paper is provided below.

Aggregation-based crystal growth and microstructure development in natural iron oxyhydroxide biomineralization products.

Jillian F. Banfield*¹, Susan A. Welch¹, Hengzhong Zhang¹, Tamara Thomsen Ebert², and R. Lee Penn³

¹ Department of Geology and Geophysics, University of Wisconsin-Madison, Madison WI 53706

² Diversions Scuba, Madison, Wisconsin 53705

³ Department of Earth and Planetary Sciences, Johns Hopkins University, Baltimore MD 21218

Crystals are generally considered to grow by attachment of ions onto inorganic surfaces or organic templates. High-resolution transmission electron microscopy of biomineralization products of iron-oxidizing bacteria revealed an alternative coarsening mechanism in which adjacent ~ 2-3 nm particles aggregate and rotate so that their structures adopt parallel orientations in 3-dimensions. Crystal growth is accomplished by elimination of

Roadmap Objectives

#18

Currently this project does not fit in an established category

water molecules at interfaces and formation of Fe-O bonds. Self-assembly occurs at multiple sites, leading to a coarser, polycrystalline material. Point defects (from surface-adsorbed impurities), dislocations, and slabs of structurally distinct material are created as a consequence of this growth mechanism and can dramatically impact subsequent reactivity.

Field Expeditions

Field work is carried out at the Piquette Mine, beneath Tennyson WI. Samples are collected by a scuba diving team who currently donate their time to the project. The university researchers involved in the work are listed above. Ms. Tamara Thomsen Ebert is the lead scuba diver, and she is a co-author on the paper included above.

Cross Team Collaborations

Future plans include collaborations with Professor Clark Johnson (UW, Madison) and Professor Dan Schrag (Harvard) for isotope work.

Project

Studies of Biogenic Greenhouse Gases on the Early Earth

Senior Project Investigator(s):

Geoffrey A. Blake

ACCOMPLISHMENTS

Research by the Blake group (as part of the NAI effort led by Principle Investigator, K. Nealson) centers on isotopic studies of biogenic greenhouse gases as evidence for life on the large scale appropriate for both remote sensing approaches and global biogeochemical investigations. Research topics over the past year have dealt primarily with early and present biogeochemical evolution of the Earth. Our efforts over the coming year(s) will focus strongly on developing new tools for the exploration of Mars and other extraterrestrial environments.

In collaboration with the Nealson and Yung groups, our recent research has involved both laboratory and observational investigation of the biogeochemistry of greenhouse gases relevant to the early Earth. Particular emphasis is placed on the quantitative examination of biologically specific tracers (such as stable isotope fractionation in these gases) and on whether or not these signatures can be reliably detected in extraterrestrial solar system bodies and exo-planetary systems. Our initial efforts have dealt with nitrous oxide (NNO). We have now characterized in detail the fractionation induced by the photolysis of NNO first predicted by Professor Yung, using a combination of non-linear spectroscopic light sources and mass spectrometry or FTIR (Fourier transform infrared reflection) spectroscopy (Rahn et al. 1998, Zhang et al. 2000, Turatti et al. 2000). We are now extending this work to include biochemical fractionation with Dr. Lisa Stein, and we are working with the Yung group to model the global biogeochemistry of nitrous oxide. Part of this effort is now funded by the NSF Atmospheric Chemistry Office through

a grant to Professor Yung. Early results made possible by NAI support were critical to this award.

Neither approach used in the lab to date is compatible with the stringent space and weight requirements of *in situ* planetary exploration strategies. We are therefore developing new technologies that should enable *in situ* measurements of abundances and stable isotope ratios in important radiatively and biogenically active gases (such as carbon dioxide, carbon monoxide, water, methane, nitrous oxide, and hydrogen sulfide) to very high precision (0.1 per mil or better for the isotopic ratios, for example). Such measurements, impossible at present, could provide pivotal new constraints on the global (bio)geochemical budgets of these critical species. They could also be used to examine the dynamics of atmospheric transport on Mars, Titan, and other solar system bodies. We believe the combination of solid state light sources with imaging of IR-LIF (infrared-laser induced fluorescence) via newly available detector arrays will make such *in situ* measurements possible for the first time. Even under ambient terrestrial conditions, the LIF yield from vibrational excitation of species such as water and carbon dioxide should produce emission measures well in excess of ten billion photons/second from sample volumes of around 1 cc. These count rates can, in principle, yield detection limits into the sub-ppt (sub-precipitate) range that are required for *in situ* isotopic study of atmospheric trace gases.

Such promising technologies are relatively immature, but developing rapidly. There are a great many uncertainties regarding their applicability to *in situ* IR-LIF planetary studies. The support from NAI was used to conduct pivotal modeling tests of this approach, which has just resulted in a successful proposal to the PIDDP (planetary instrument definition and development program) with its funding start in May 2000. PIDDP now provides the larger funding base to fabricate these new tools through a three-year program. Tool development includes these endeavors: (1) combine microchip near-IR lasers with low background detection axes and state-of-the-art HgCdTe detectors developed for astronomical spectroscopy to investigate the sensitivity of IR-LIF under realistic planetary conditions; (2) optimize the optical pumping and filtering schemes for important species; and (3) apply the spectrometer to non-destructive measurement of stable isotopes in a variety of test samples.

These studies form the necessary precursors to development of compact, lightweight stable isotope/trace gas sensors for future planetary missions. Within the NAI consortium, we will continue to work with the Nealson and Yung groups on modeling of: (1) the potential biological activity on Mars and the early Earth; and (2) the isotopic signatures of various model biospheres. This research is groundbreaking. It provides the necessary context for IR-LIF to be used as a robust diagnostic of extant or extinct biota (through its sampling of gases trapped in Martian soils and ice caps) on and underneath planetary surfaces.

HIGHLIGHTS

Cross Team Collaborations

We are working with members of the Nealson group, in particular Dr. Lisa Stein, to investigate the biological stable isotope fractionation introduced in the synthesis and con-

Roadmap Objectives

#18

Currently this project does not fit in an established category

sumption of nitrous oxide. As new *in situ* stable isotope instruments come on line, we expect this collaboration to grow and to extend to other members of the NAI team.

Project

Origin of Martian Soils

Senior Project Investigator(s):
Diana L. Blaney

ACCOMPLISHMENTS

RATIONALE

The Martian bright soils seem to be compositionally homogeneous and globally distributed. Also, they have many unusual elemental, magnetic, and mineralogical properties that are related to their formation environment. As these bright soils are volumetrically the largest soil unit on Mars, their formation mechanism is central to understanding Martian climate evolution and the relative role of various geologic processes in altering the Martian surface.

PROGRESS

Examination of existing data shows that Martian bright soils are poorly crystalline and show no evidence for carbonates or clay minerals. This may imply that the dominant weathering processes on Mars did not involve significant amounts of liquid water and casts doubt on the hypothesis that Mars may have been warm and wet for an extended period of time. If Martian climate did not have a clement period, then the environments where life may have thrived in the past may be very limited. We are in the process of revising a manuscript on this topic.

Weak absorption features in the 2-2.5 mm region have been interpreted as clays and may imply prolonged aqueous activity. However, the interpretation of these absorption features is complicated by the solar, Martian, and Earth atmospheres. New higher spectral resolution data collected at Kitt Peak will permit us to separate out the narrower atmospheric features from the broader mineral features on the surface. This will enable us to determine if crystalline clays are present in Martian bright soils.

SCHEDULE

We did not meet our goal of publishing the Mars soil paper this year, but we expect to have it done by the end of the summer.

FUTURE WORK

We will publish the work on Martian soil evolution outlined in the Blaney (1999) abstract regarding geochemical aspects of Martian soils. Also, we will continue with the interpretation of new telescopic data and assess its impact on models of Martian soil formation.

Isotopic Biosignatures of Bacteria

Project

Senior Project Investigator(s):
Marilyn L. Fogel

ACCOMPLISHMENTS

Fogel and Ziegler established the isotopic biosignatures of bacteria and how these organisms transform primary (in this case photosynthetic) isotopic and protein signals into altered microbial biosignatures. We found that bacteria totally resynthesized and scrambled the isotopic patterns in amino acids. The process was rapid for soluble proteins, e.g., Rubisco. Bacteria proteins were mineralized and recycled as well. Our data show that on very short time scales (hours to days to months), microbial products are formed and degraded. The biologically-resistant material gradually obtains an isotopic and chemical composition that has little resemblance to original biochemical compounds.

Bacteria from the Archae and the Eubacteria were cultured at the Geophysical Laboratory by James Scott or at the American Type Culture Collection in collaboration with David Emerson. The work is continuing on several fronts. James Scott is actively seeking to determine the carbon isotope fractionation during acetogenesis. He has examined cultures of acetogens that have been grown heterotrophically and found that the cellular carbon is derived from components in the medium without major isotopic fractionation. Autotrophic cultures are being presently grown. More individual amino acid isotopic analyses are planned for the cultured microorganisms.

We used SELDI (surface enhanced laser desorption ionization) technology to determine the nature of high molecular weight organic molecules found in oceanic and aquatic dissolved organic matter (DOM) to see whether we could detect large proteins or peptidoglycan fragments. Results of these experiments are as follows:

- The molecular weight distribution of the samples was in the range of 800 to 6,000 daltons. No material was detected between 10,000 and 100,000 daltons.
- A set of fourteen peaks was found in both the riverine and oceanic DOM.
- The riverine sample had the highest molecular weight peak at 6,000 daltons. This broad peak indicated a glycoprotein or other carbohydrate-bound moiety.
- The oceanic DOM sample had a series of molecular fragments in the range of 1,200 to 2,300 daltons that were unique to this sample.
- Both samples had a suite of peaks that corresponded to a class of compounds with subunits in the range of 200-230 daltons.

HIGHLIGHTS

Field Expeditions

Field trips were taken to the Jug Bay Wetlands Sanctuary, a preserve on the Patuxent River in Maryland. We also analyzed samples from Antarctica (collected by Sun and

Roadmap Objectives

#5

Linking Planetary & Biological Evolution

#6

Microbial Ecology

#7

Extremes of Life

Roadmap Objectives

- #3 Models for Life
- #5 Linking Planetary & Biological Evolution
- #8 Past and Present Life on Mars
- #11 Origin of Habitable Planets
- #13 Extrasolar Biomarkers

Friedmann) and Mono Lake samples (collected by the JPL group).

Cross Team Collaborations

I have collaborated with the Carnegie Institution team and the JPL group.

Project

Fe Isotope Biosignatures

Senior Project Investigator(s):

Clark M. Johnson, PI

Co-Investigator:

Brian L. Beard

ACCOMPLISHMENTS

Our initial premise for using Fe isotopes as a biosignature was that the relative mass differences of four Fe isotopes (^{54}Fe , ^{56}Fe , ^{57}Fe , and ^{58}Fe) would be sufficiently small (such that inorganic processes would produce minimal fractionations), while biologic (metabolic) processes would produce measurable fractionation outside the "noise level" of abiologic effects. In terms of nomenclature used to describe Fe isotope variations, we define:

$$\Delta^{56}\text{Fe} = \left[\frac{{}^{56}\text{Fe}/{}^{54}\text{Fe}(\text{SAMPLE})}{{}^{56}\text{Fe}/{}^{54}\text{Fe}(\text{EARTH-MOON})} - 1 \right] 10^3$$

where the reference value is the $^{56}\text{Fe}/^{54}\text{Fe}$ ratio of the bulk Earth and Moon (Beard and Johnson, 1999).

Our results obtained in the initial phase of our NAI-funded research has clearly demonstrated the great potential that Fe isotopes have for a robust biosignature. Much of our work in the first year of NAI-funding focused on developing the analytical methods required for high-precision Fe isotope analysis (Johnson and Beard, 1999). Our work to date has shown:

- There is a constant and apparently homogeneous Fe isotope "baseline composition" for igneous rocks from the Earth and Moon (Figure 1; Beard and Johnson, 1999).
- Fe-reducing bacteria produce a sustainable isotopic fractionation, where the $^{56}\text{Fe}/^{54}\text{Fe}$ ratio is ~ 1.3 ‰ lighter than that of ferric oxide substrate, indicating a clear biological fractionation (Figure 1; Beard et al., 1999).
- Fe isotope compositions of sedimentary rocks that may have had a biologic component to their genesis record Fe isotope variations on the order of 1-2 ‰, similar to that observed in biologic experiments (Figure 1; Beard and Johnson, 1999; Beard et al., 1999).

In addition, we have started looking at modern microbial systems (groundwater stud-

ies) to evaluate the time scales and preservation potential of Fe isotope biosignatures. Fe(II) groundwater plumes have consistently low $\delta^{56}\text{Fe}$ values over periods of one year, and biogenically-deposited Fe-oxides have similarly low $\delta^{56}\text{Fe}$ values (Figure 1). In parallel with these NAI-funded projects, we have started a detailed study of Banded Iron Formations (funded by NSF), where large variations in $\delta^{56}\text{Fe}$ values have been found that may be related to biologic activity. We have also looked at Fe-Mn nodules (not to be confused with the crusts analyzed by Zhu et al., 2000), which may form by mobilization of sediment iron by Fe-reducing bacteria. The isotopic compositions of large nodules are strikingly similar to those produced by Fe-reducing bacteria in the lab (Figure 1).

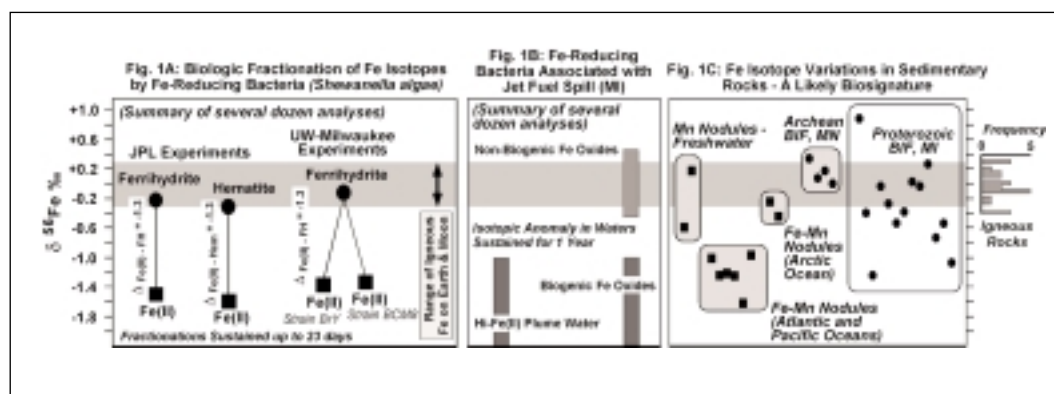


Figure 1

Summary of Fe isotope studies conducted at U.W. Madison (Beard & Johnson, 1999; Beard et al., 1999; Johnson and Beard, 1999; Beard, Johnson, Skulan, Nealson, Cox, Sun, & Gerdinech, unpublished data).

An additional effort in the first two years of NAI work has been construction of a new laboratory and accompanying instrumentation. These facilities will enable us to pursue Fe isotopes at a much faster rate and at significantly greater precision than any other laboratory in the world. In 1999, we completed the funding package required for purchase of a next-generation Multi-Collector, Inductively-Coupled Plasma Mass Spectrometer (MC-ICP-MS) that is ideally suited for ultra-high-precision analysis of Fe isotopes. This \$800K instrument is now installed in a new \$100K clean lab at U.W. Madison. Funding for this \$900K project came from U.W. Madison (\$400K), the NASA Astrobiology Institute (\$250K), and the National Science Foundation (\$250K). The new MC-ICP-MS we have now installed at U.W. Madison is the only instrument capable of removing common molecular isobars on the Fe mass spectrum, including these three entities: (1) $^{40}\text{Ar}^{14}\text{N}$ on ^{54}Fe ; (2) $^{40}\text{Ar}^{16}\text{O}$ on ^{56}Fe ; and (3) $^{40}\text{Ar}^{16}\text{OH}$ on ^{57}Fe . Past work using first-generation MC-ICP-MS (e.g., Zhu et al., 2000; Anbar et al., 2000) attempted to minimize interferences by analyzing very large samples (up to 20 ppm), and not all Fe isotopes were measured. Our new NAI-funded MC-ICP-MS instrument removes the Argide isobars, allowing us to make ultra-precise Fe isotope measurements (with a factor of five higher precision than any other lab) on all of the Fe isotopes, with a minimum of sample consumption (50-100 ppb solutions; up to three orders of magnitude lower than any other lab).

Finally, we have begun an exhaustive set of inorganic and abiologic experiments to: (1) determine the nature and extent of abiological processes that can fractionate Fe isotopes; and (2) assess the possibility that these effects could be preserved in the rock record. Such work is clearly required to establish Fe isotopes as a unique biosignature. These experiments also serve as abiological controls for our biological experiments. This work is also important in light of three recent studies that have either: (1) criticized our proposal that Fe isotopes may be a robust biosignature (Zhu et al., 2000; Anbar et al., 2000); or (2) proposed significant inorganic isotopic fractionation (Polyakov and Mineev, 2000). If these criticisms and studies are valid, they would significantly reduce the power of Fe isotopes as a biosignature.

Much of the "debate" on applying Fe isotope variations as a biosignature stems from confusion over kinetic versus equilibrium issues. Biologic or "vital" effects might best be thought of as "sustained kinetic fractionations" (where metabolism or other biologic effects supply the driving force that keeps the system from obtaining thermodynamic equilibrium). We interpret our data for *Shewanella* algae as a metabolically-sustained kinetic isotope fractionation, which we have measured in runs up to 23 days in length (Figure 1). Longer-term (~ one year) isotopic anomalies have been measured for Fe(II) groundwater plumes that are sustained by Fe-reducing bacteria (Figure 1). Such long-term kinetic fractionations are likely to be preserved in nature, which, of course, is an essential component for a biosignature.

Recent criticism of Fe isotopes as a biosignature has used apparent Fe isotope fractionations of several per mil on ion-exchange columns (used to separate Fe chemically from complex natural samples) to argue that similar ranges in Fe isotope compositions may occur abiologically in nature (Anbar et al., 2000). Unfortunately, Anbar et al. (2000) did not realize that they did not attain isotopic equilibrium in their system, which has been known to be a major issue in ion-exchange columns since the 1930's. Our work has shown that attainment of isotopic equilibrium is never achieved at the high flow rates used by Anbar et al. (2000). Lack of isotopic equilibrium produces strong vertical isotopic gradients in the columns that do not come to isotopic equilibrium even after elution of 99% of the Fe. We contend that such extreme and short-term kinetic isotope fractionations are unlikely to be seen in nature. They would therefore not give a "false positive" biosignature.

We summarize the results of our inorganic experiments to date in Figure 2. These results are organized by time scale, emphasizing the need to separate equilibrium, long-term kinetic, and short-term kinetic fractionations in regard to application of Fe isotopes as a biosignature in nature. Our working hypothesis is that while short-term kinetic Fe isotope fractionations are interesting, they have little applicability to nature. Of more importance to using Fe isotopes as a biosignature are the: (1) long-term, biologically-sustained Fe isotope anomalies we have measured in the lab and in natural groundwater systems; and (2) equilibrium Fe isotope fractionations in inorganic systems. Note that although we find a short-term (hours) kinetic Fe isotope fractionation during acid-hydrolysis of hematite, under equilibrium timescales (months), there is no measurable equilibrium isotope fractionation. These results counter recent proposals that abiologic Fe isotope fractionation is important in nature, and they confirm our initial premise that Fe isotopes may uniquely identify life.

Year 2

For the future, we will continue our biological experiments with the JPL group, including new experiments on Fe-oxidizing bacteria. In addition, we have a comprehensive plan for additional abiologic and inorganic experiments.

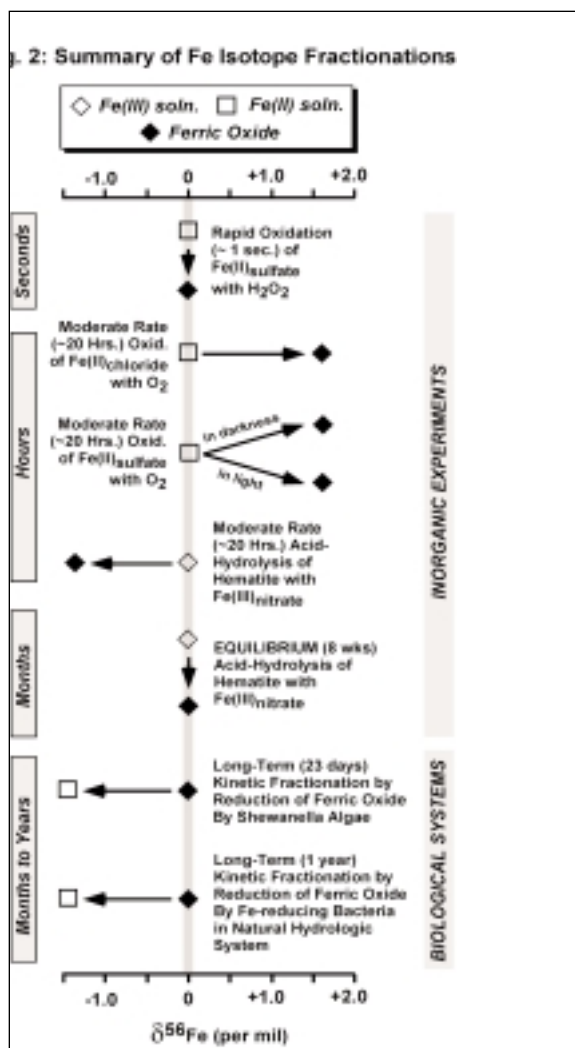


Figure 2

Summary of kinetic and equilibrium Fe isotope fractionations for inorganic systems, as well as microbial systems in the lab and nature. Note that the effect of kinetic isotope fractionations on the use of Fe isotopes as a biosignature depends upon the timescales involved.

HIGHLIGHTS

- We have shown, for the first time, that Fe isotopes are fractionated by Fe-reducing bacteria. This finding contrasts with the apparent isotopic homogeneity of inorganic (igneous) Fe.
- Preliminary abiologic experiments show short-term kinetic isotope fractionations, but no equilibrium abiologic fractionations. This suggests that Fe isotopes may uniquely identify metabolic processes in the rock record. We are currently evaluating inorganic fractionations in greater detail.

Roadmap Objectives

#6

Microbial Ecology

#7

Extremes of Life

#8

Past and Present Life
on Mars

#9

Life's Precursors &
Habitats in the Outer
Solar System

#10

Natural Migration of Life

#11

Origin of Habitable
Planets

#12

Effects of Climate &
Geology on Habitability

#14

Ecosystems Response to
Rapid Environmental
Change

#17

Planetary Protection

Cross Team Collaborations

Our major collaborations have been with Ken Nealson's JPL team. In addition, we have arranged for collaborative work with the UCLA group (Archean microfossils with Bill Schopf) and the Penn State group (Archean paleosols with Hiroshi Ohmoto). This work will be done in the coming year.

Project

Coevolution of Life and Planets

Senior Project Investigator(s):

Joseph L. Kirschvink

ACCOMPLISHMENTS

The Kirschvink lab has focused on several aspects of interest to the NASA Astrobiology Institute during the past 1.5 years, including: (1) evaluation and identification of Precambrian biosignatures; (2) testing the Panspermia hypothesis with the Martian Meteorite ALH84001; (3) assessing the effect of the Paleoproterozoic 'Snowball Earth'; and (4) placing constraints on study of life on Europa.

Biosignatures

Our most important work in this area has been working on the morphology of the fine-grained magnetite in the carbonate blebs of the Martian meteorite ALH84001, in collaboration with Kathy Thomas and Dave McKay of the Johnson Space Center. In this meteorite, 27% of the magnetite crystals are indistinguishable from the magnetite produced by living magnetotactic bacteria. This is the strongest evidence yet for life on Mars, and the combination of 6 biosignatures has no known mechanism formed through inorganic processes. Indeed, there are fundamental chemical and physical reasons why inorganic origins are implausible. (Thomas-Keprta, Wentworth et al., 1999; Thomas-Keprta, Bazylnski et al., 2000)

We are also developing two additional techniques for biosignature detection with groups at JPL. These include: (1) an *in situ* chemical imaging system that combines AFM (atomic force microscopy) laser-ablation chemical spectroscopy (work with Greg Bearman at JPL); and (2) a microwave technique for detection of elongate magnetofossils (work with Sam Kim at JPL).

Panspermia

On a related project spearheaded by Ben Weiss, we have demonstrated that ALH84001 moved from Mars to Earth without ever reaching temperatures of 40°C. Hence, this is the first evidence that life can jump between planets. Life on Earth may have evolved first on Mars, then jumped here. (Weiss, Kirschvink et al., 2000; Weiss, Kirschvink et al., 2000)

Life on Europa

Using straightforward arguments concerning redox gradients and the source of oxidants, we have shown that any biosphere on Europa would be severely limited by lack of meta-

bolic energy. Don't expect fish, but you might find an occasional microorganism (Gaidos et al., 1999).

Snowball Earth

We have discovered that the Kalahari manganese field in South Africa formed in the immediate aftermath of one of the most severe episodes of global glaciation in Earth history, ~ 70 Myr in duration (Kirschvink et al., 2000). We are currently testing a hypothesis that the Ongeluk flood basalts (immediately overlying the tillite) may have been triggered by lithospheric contraction produced by a prolonged episode of refrigeration on the continental crust (Raub et al., in preparation).

Nearby Young Solar Analogs

Eric Gaidos has been studying a catalog of young solar-type stars within 25 pc as plausible analogs to the astrophysical environment of the Sun and the Earth during the first 800 Myr of solar system history. We are carrying out a multi-wavelength (from X-rays to radio waves), multidisciplinary, and collaborative campaign of observations and analysis to study these objects.

Metazoan Radiation & the Cambrian Explosion

For the last year and a half, Whitey Hagadorn has been a postdoctoral scholar in the Kirschvink laboratory. During this interval, he has been working on a number of projects that relate to astrobiological research. Primary emphasis is on: (1) examining the paleobiological record of the terminal Neoproterozoic; and 2) studying the response of metazoans to major perturbations in Earth's history, such as the late Neoproterozoic Marinoan glaciations and the earliest Cambrian biomineralization event. Some of the current and planned projects are outlined below. (See Projects 1-4 immediately below.) Following the projects is a list of astrobiology-related papers, which he has published or submitted in the last 1.5 years.

Current and planned projects

Whitey Hagadorn Report

Project 1

Noninvasive 3-D Visualization of Bacteria in Extreme and Precambrian Earth Rocks:
A Prelude to Martian Sample Analysis

Collaborators

J. Kirschvink (CIT), K. Nealson, A. Tsapin (JPL)

Questions/Objectives

- Employ microscopic computerized tomography (microCT) as a primary noninvasive search mechanism for identification of bacteria embedded in rocks.
- Refine protocols for extracting and rendering three dimensional models of filaments from both matrix-embedded Precambrian samples and in modern samples bearing endolithic cyanobacteria.

Description

The search for direct evidence of life on other planets and in early Earth history requires

analysis of solid objects, such as rock or soil samples. Bacteria from extreme Earth environments (e.g., dry valleys, hydrothermal springs) provide a glimpse of how life might manifest itself on other planets. Fossilized microbiotas from Earth provide a glimpse of how the remnants of such life might be preserved in extraterrestrial samples. Although a variety of geochemical, spectroscopic, mineralogic, and related techniques are being developed to assess extant and fossil life signatures, there are few noninvasive, non-destructive techniques being developed to probe samples and identify areas of potential investigation.

Recent technological advances in microCT technology allow penetration of highly dense samples and identification of features with 5mm precision, thus providing a means for preliminary scouting of both terrestrial and extraterrestrial samples for morphologic evidence of life. In pilot microCT studies of silicified Neoproterozoic microfossils, we find lithified microfossils >10 mm in size are clearly visible. Similarly, living endolithic algae, fungi, and lichens are visible in quartzitic samples from Antarctic dry valleys. At present, we plan to write about these preliminary results this summer, and we will present our findings at the Fall Meeting of the Geological Society of America.

Given that the resolution levels of microCT will approach 1 mm in coming years, it is logical to develop a set of protocols for using this technique to analyze extraterrestrial rock and soil samples. Archean and Proterozoic microbiota samples and modern endolithic bacterial samples will be analyzed using microCT. In addition to quantification of the location and morphometric characteristics of biotas within each sample, protocols will be developed for isosurface mapping and volume rendering individual fossils. A spectrum of preservational and lithic types will be analyzed in order to: (1) identify which sample types are most amenable to these noninvasive scouting techniques; and (2) provide a noninvasive prelude to subsequent invasive and destructive biological and geochemical analyses.

Project 2

Paleobiology, Paleoecology, and Taphonomy of Neoproterozoic Megafaunas: Morphologic, Microfabric, and Microbial Insights

Collaborators

D. Breen (CIT), B. Waggoner (UCA), J. Nesson (NIH)

Questions/Objectives

- Is Ediacaran preservation and ecology tied to microbial binding of siliciclastic substrates?
- How do preservational variations impact our understanding of their morphology?
- Do putative metazoan Ediacaran fossils contain a gut and/or coelom?
- Is the first appearance of metazoans tied to this unique preservational scenario, or is it linked to evolutionary novelty developed in the post-Snowball aftermath?

Description

Preservation of Ediacaran organisms in siliciclastic sediments is hypothesized to have been restricted by microbial mantling of carcasses, coupled with subsequent restriction of pore-water migration within such microbial mat-laden sediments. Because Ediacaran

preservation is unlike that of other Phanerozoic or modern environments, numerous difficulties exist in interpreting their paleobiology and paleoecology. This leads to debate whether some Ediacarans are animals, lichens, and/or constitute their own Kingdom.

To test the hypothesis that preservation of Ediacaran faunas is mediated by microbial mantling, the first phase of this project will utilize detailed microfabric, geochemical, SEM (scanning electron microscopy), and x-radiographic analyses to identify diagnostic microbial structures (e.g., wrinkle structures, "sponge sand" textures, biomarkers, $\Delta^{13}\text{C}$ org signatures) in ancient Ediacaran-bearing samples. Phase two of this project will involve CT-scanning (computed tomography-scanning) of Ediacaran fossils from both fine- and coarse-grained deposits to obtain quantitative morphologic characteristics for each taxon. Volumized density isosurfaces will be rendered to evaluate postburial morphology of matrix-embedded samples. These volumes will provide an initial datum for retro-deformation of fossil models into possible preburial morphologies. Phase three will involve evaluation of density and microfabric variations within Ediacaran fossils preserved in fine-grained strata. This is done in order to identify internal features within the fossils, which might provide clues about their anatomy and placement within existing clades (e.g., subtle variations in composition, porosity, and sedimentologic features possibly associated with preferential decay of soft tissue, including coelomic cavities).

To date, I have been trained in operation of medical CT (computerized tomography) equipment, and I have worked to streamline data transfer from medical formatting to cross-platform formats. At present, I am learning to utilize volume rendering and iso-surface mapping software. We have pilot data sets for a variety of Ediacaran biotas and faunas from other fossil Lagerstätten. These comprise a range in preservational and lithologic modes. In several cases, we have identified concealed features with the potential to elucidate the taxonomic affinity of some of these organisms. For example, analyses of several discoidal forms (e.g., Cyclomedusa, Nimbria, Spriggia, Tirasiana) quickly allows separation of discoids into paleobiologically meaningful groups that share morphologic synapomorphies, such as presence of a stalk or an internal cavity.

Project 3

Significance, Taxonomy, and Biomineralization of Terminal Neoproterozoic–Lower Cambrian Conical Fossils

Collaborators

J. Kirschvink (CIT), W. Fritz (GSC), J. Hollingsworth (Union Carbide), F. Corsetti (UCSB)

Questions/Objectives

- Do problematic conical fossil concentrations (e.g., Cloudina coquinas) represent the earliest "disaster taxa"?
- Are these morphologically similar, but simple, faunas related?
- How did biomineralization develop in the earliest skeletal fossils?
- Is the onset of biomineralization related to post-"Snowball Earth" changes in ocean chemistry, and/or exaptation of an ancestral biomineral system?

Description

Fossil concentrations (or "shell beds") dominated by problematic conical taxa (e.g.,

Cloudina, Volborthella, Salterella, Orthothecida) are a relatively common type of shell bed in Terminal Neoproterozoic–Lower Cambrian strata. They are particularly extensive in strata of the southwestern Great Basin and northwestern Mexico. Because most of these fossil concentrations are intimately associated with hiatal surfaces and trilobite extinction events, two research hypotheses exist: (1) these conical fossil concentrations are hypothesized to represent the earliest opportunistic "disaster taxa"; and (2) coeval Cloudina beds of varying mineralogy may provide insights about the development of metazoan biomineralization.

Our research studies these two specific areas:

(1) To test the "conical fossils as disaster taxa" hypothesis, the paleoenvironmental distribution, mode of deposition, and stratigraphic position of these conical fossil deposits will be characterized. Conical fossil concentrations will be examined in detail in the field using standard shell-bed analytic, sequence stratigraphic, and biostratigraphic techniques to determine: (a) if they reflect opportunistic disaster taxa; (b) what factors might account for their abundance (i.e., eustatic, geochemical, predation factors); (c) what ecologic niches might be compatible with their rapid appearance; and (d) if or how such horizons may be utilized as regional biostratigraphic cues in Project 4 below.

(2) Based on the best preserved silicified and phosphatized samples, various techniques (petrographic, microCT, microprobe, and geochemical) will be used to identify the locus and nature of biomineralization within "protoconches" of Cloudina. Additionally, such techniques will be used to evaluate the relationship between variations in skeletal mineralogy observed within: (a) coeval cloudiniid concentrations; (b) regional patterns in oceanic $^{87}\text{Sr}/^{86}\text{Sr}$ and $\Delta^{13}\text{C}$ carb; and (c) other biomineralization events. Mineralogic, morphologic, and related contextual information will provide a logical first step toward identifying characters to evaluate both the systematics of these conical fossil taxa, as well as their role in the development of biomineralization within the Metazoa.

To date, we have identified and collected pilot samples from most of the major conical fossil horizons in California, Nevada, and Mexico. A rough biostratigraphic framework has been constructed to evaluate the synchronicity of putative coeval conical fossil concentrations. MicroCT analyses of silicified cloudiniids have yielded the best morphometric information to date, and petrographic analysis of these pilot samples will occur this summer.

Project 4

The Terminal Proterozoic–Cambrian Transition: Southwestern North America

Collaborators

J. Kirschvink (CIT), B. Wernicke (CIT), F. Corsetti (UCSB), J. Stewart (USGS), R. Amaya Martinez (UNAM)

Questions/Objectives

- Create a 20 Ma-precision and integrated geochronologic, biostratigraphic, paleomagnetic, lithostratigraphic, and geochemical framework for Neoproterozoic strata of southwestern North America.

- Based on this framework:

Are strata of the Sonoran province correlative to Precambrian-Cambrian strata of the southwestern U.S.?

Do glaciogenic diamictites and associated "cap carbonates" trigger the onset of metazoan evolution?

Is the appearance of metazoans really linked to rapid inertial interchange (true polar wander)?

Description

One of the most intriguing areas of modern stratigraphic research centers on the sudden diversification of multicellular life and its relationship to global environmental changes at the end of the Proterozoic. Debate persists regarding the timing and number of glaciations during this interval, global changes in ocean chemistry reflected in isotopic excursions, the timing and nature of true polar wander, and how these events relate to the Cambrian radiation and its precursors.

Southwestern North America contains a well-exposed, relatively complete Neoproterozoic-Cambrian succession that is well characterized lithostratigraphically. It contains well identified glacial intervals, abundant carbonate for chemostratigraphic analysis, high potential for biostratigraphic preservation, locally well-preserved Proterozoic magnetizations, and good potential for chronostratigraphic control. Given recent advances in dating diagenetic minerals in non-volcanic clastic strata, the succession therefore has strong potential for: (a) constraining the total number of terminal Proterozoic glacial events (and associated "cap" carbonates); (b) identifying their relationship to isotopic excursions; and (c) assessing any relationship between these events and the diversification of phytoplanktic and/or multicellular life.

To evaluate the relationship between these events, we will establish an integrated bio-, chrono-, chemo-, and magnetostratigraphic framework for Neoproterozoic to Lower Cambrian strata in southwestern North America. This framework will link the two main regional Proterozoic-Cambrian successions, including strata in the White-Inyo/Death Valley/Mojave and the Sonora geographic areas. My contribution to this research effort will be constructing the microbiota and macrofossil biostratigraphic framework, analyzing diagenetic mineral assemblages to construct a geochronologic framework, and evaluating the relationship between hypothesized snowball and true polar wander events to biotic shifts.

HIGHLIGHTS

Field Expeditions

Kirschvink & Weiss spent six weeks (October/November 1999) collecting samples related to the Paleoproterozoic 'Snowball Earth' episode in the Kalahari desert and to the Permian/Triassic extinction in the Karoo Desert (Southern Africa). Kirschvink, Weiss, and MacDonald also spent two weeks sampling the P/T and Triassic/Jurassic boundary in central Japan.

Roadmap Objectives

#1

Sources of Organics on Earth

#7

Extremes of Life

#8

Past and Present Life on Mars

To date, Whitey Hagadorn has spent one field season in Mexico, in which he examined one section in nearly every major outcrop area of Mesoproterozoic-Cambrian strata in Sonora. Pilot samples have been collected from this region for paleobiologic, geochronologic, and chemostratigraphic analyses. Pilot samples have also been collected from the Death Valley and White-Inyo regions of California-Nevada. From these pilot samples, acritarch-bearing, microfossil-bearing, and xenotime-coated-zircon-bearing samples have been identified. This fall and winter, Hagadorn plans to continue field work in these regions and resample the most fruitful sections in detail for further analyses.

Cross Team Collaborations

Working with the Bowring group at MIT, we recently reported a critical age constraint for the origin of the first bilaterian fossils.

Publication about this work: Martin, M.W., Grazhdankin, D.V., Bowring, S.A., Evans, D.A.D., Fedonkin, M.A., and Kirschvink, J. L. (2000). Geochronological constraints of the Late Vendian body and trace-fossils, Zimmie Gory, White Sea, Russia: Implications for Metazoan evolution in the Neoproterozoic. *Science*, 288: 841-845.

Project

Organic Molecules as Biosignatures

Senior Project Investigator(s):

Gene D. McDonald

ACCOMPLISHMENTS

Our several research projects in progress are related to the issue of identifying and evaluating biosignatures. We have collected a set of data on the fatty acid composition of Mono Lake water and sediments. These data indicate that the level of early diagenesis in the lake is relatively low, thus preservation of organic biomarkers should be enhanced. We are following up on this work with analysis of fatty acid carbon isotope signatures, in collaboration with Drs. Marilyn Fogel and James Scott of the Carnegie Institution of Washington.

We are also studying the temperature and environmental history of Siberian permafrost as revealed by the rate of amino acid racemization in permafrost core samples provided by Dr. David Gilichinsky of Russia. This study will allow us to evaluate more thoroughly the claims that viable bacteria, isolated from permafrost several million years old, have remained dormant without significant warming during their entire residence in the sediment. Data obtained so far indicate that the temperature of permafrost located below approximately 10 m has been relatively constant over time.

Another project currently underway involves the transport of organic material produced by cryptoendolithic organisms that colonize the interiors of Antarctic rocks. Soluble

organics, including amino acids, appear to be transported into the abiotic interior of these rocks by small flows of condensed atmospheric water. Amino acids detected in these rock interiors so far show significant amounts of racemization. This indicates that they have been in the interior for long periods of time and that the interior is free of living organisms. This environment represents perhaps the only place on Earth where potential organic biomarkers remain in contact with mineral matrices for extended periods of time without alteration by active biology.

Determination of rate constants for oxidation of organic macromolecules by possible Martian oxidants is another continuing research project. We have collected a data set consisting of rate constants for the oxidation of humic acid by dry hydrogen peroxide as a function of temperature. We are now incorporating these data with Martian annual maximum and minimum temperature data furnished by Prof. Bruce Jakosky of the University of Colorado.

We continue our research work on application of multivariate statistical techniques to the analysis of organic biomarker data from extraterrestrial samples. This study is currently focused on developing neural net algorithms that can distinguish between biological and non-biological amino acid profiles in meteorite or sediment samples.

Finally, we have in the past two years continued analyses of the amino acid content of Martian meteorites, most recently that of the meteorite Nakhla. The amino acid profile of this meteorite appears to be primarily terrestrial in origin, consistent with those of EETA79001 and ALH84001 previously analyzed.

Martian Environments

Project

Senior Project Investigator(s):
Jeff Plescia, Larry Soderblom

ACCOMPLISHMENTS

The focus of the work has been on examining aspects of the Martian environment that are relevant to Mars as a possible habitat for prebiotic or biotic activity. This work has examined the modern and the ancient Martian environments.

In terms of modern Mars, it is clear that the current environment is not conducive to the preservation of organic matter at the surface because of the presence of an oxidant and the high ultraviolet flux. Therefore, if such organic materials existed in the past, they would only be preserved in the subsurface or in material recently brought to the surface. Finding recently-exposed material is therefore of high importance. There are two mechanisms that can expose subsurface material at the surface: impact cratering and landslides.

We have examined the Mars cratering rate to estimate the number of craters and the depth of material excavation as a function of time. For example, the estimated number of 1 km craters, which would both expose material from depths of up to 150 m and be <100,000 years old, ranges from about 10-30, depending upon the flux model chosen. These estimates suggest that finding fresh craters is an important mission planning priority.

Examining landslides within Valles Marineris is a study area considering two possibilities. Is the Mars landslide process continuous with new wall exposures continually formed, or does a Mars landslide formation occur only at a single point in time? TES (Thermal Emission Spectrometer) data are also being examined to determine the composition of the material exposed in the walls of Valles Marineris and in layered deposits of the interior.

Volcanic terranes on Earth provide environments for a variety of thermophilic organisms. Such terranes on Mars might also be suitable habitats. Early in Martian history when the impact flux was high and planetary scale sterilization events were occurring, the subsurface would have represented a protected environment. The margins of the Hellas Basin (southern hemisphere of Mars) are areas of volcanism and large-scale fluvial processes. The spatial association of heat and water in that region suggest that subsurface hydrothermal systems would have been established there. Given the scale of the basin and the volcanism, such systems would have been long lived. These environments might have been locations for the development and sustenance of thermophilic organisms. Modern areas of volcanic and fluvial activity include the Cerberus Plains. This region has had large-scale flood basalt eruptions and is also very young (106 million years). The Cerberus Plains could be an area where thermophilic organisms could have found a modern niche.

Examination of the layered deposits of the Mars North and South Pole regions using MOC (Mars Orbiter Camera) images is underway. It is being done to determine if the age of Mars material can be better constrained. Using MOC images, very small diameter craters can be observed. Since these are more abundant than large diameter craters, age variations might be reflected in their spatial variations. Relatively few small diameter craters are observed, which is consistent with the very young age of the layered material.

We continue to examine aspects of the valley networks and erosion characteristics of Mars using MOC and MOLA (Mars Orbital Laser Altimeter) data. There is a significant discrepancy with respect to development of the channels and the absence of fine scale surface features indicative of surface flow.

HIGHLIGHTS

Cross Team Collaborations

Collaborations with Dr. Jeff Plaut (JPL) involve study of issues related to impact cratering on Mars.

CoEvolution of Planets and Biospheres: Lessons From Earth and Mars

Project

Senior Project Investigator(s):
Yuk L. Yung

ACCOMPLISHMENTS

Current Research Summary:

Creation of a Mobile Field Laboratory for *In Situ* Measurement of Biogenic Gas Fluxes

A transportable laboratory was created to: (1) concentrate gases evolving from the surface of a microbial mat; (2) measure the physical and chemical changes that occur at the mat surface over a diurnal cycle; and (3) quantitatively and sensitively measure a large variety of gases in real time. A gas-tight plexiglass chamber that could be placed on the mat surface to concentrate evolved gases was devised. Gas-tight ports were positioned on top of the chamber to drop and hold instruments at the mat surface for measuring changes in pH, temperature, and conductivity. A light meter was attached to the top of the chamber. Two battery-operated fans on the inside of the chamber insured mixing of the gases, and a teflon-lined septum was attached to the side of the chamber to collect samples. A gas chromatograph (GC) was configured with three columns and four detectors for the quantitative measurement of a large variety of fixed gases, sulfur gases, and gases containing organic carbon. The GC was calibrated with standard samples of CO₂, CH₄, NO₂, H₂S, DMS, and SO₂ in the laboratory. A system for collecting gas in the field was tested in the laboratory using evacuated vials and sterile syringes. Once in the field, all of the instrumentation was powered by a small gas generator.

Collection of Data at Field Site

Our field study site was located in Baja California, Mexico, approximately 100 miles south of Ensenada. The microbial mat communities were only a few millimeters thick, due to their destruction during the last two El Niño events. The flux chamber was placed over a section of mat that had four visible layers: (1) a saline crust on top; (2) a dark green layer dominated by cyanobacteria; (3) a brown layer containing a mixture of anoxygenic photosynthesizers and anaerobic bacteria; and (4) a black sulfidic mud layer that was at least a meter deep.

Three field trips were made for this research: one in September 1999, one in November 1999, and one in February 2000. In the September trip after testing the field equipment, we collected temperature, light, and pH profiles over an 8-hour period, then we made measurements of two sulfur gases over a 5-hour period. In November, we collected data over two consecutive days for 13-hour time periods: 9 hours in daylight and 4 hours in darkness. We observed the flux of 5 gases (relative to ambient concentrations) that correlated with temperature inside of the flux chamber. Two sulfur-containing gases, CO₂, and two organic carbon-containing gases were monitored. One of the sulfur gases was likely H₂S, based on the retention time of H₂S standards. The identities of the other

Roadmap Objectives

#1

Sources of Organics on
Earth

#5

Linking Planetary &
Biological Evolution

#6

Microbial Ecology

#7

Extremes of Life
on Mars

gases are unknown. Light, temperature, and pH profiles were also collected. The conductivity of fluid at the mat surface was too high for our instrument to measure. The pH remained circum-neutral throughout the diurnal cycle, with slight fluctuations toward alkalinity during high light-flux periods. In the February trip, we attempted to repeat the experiments from November, but we experienced difficulties with our instrumentation. The data from November are currently being transformed into measurements of gas flux per unit area per unit time for use in atmospheric modeling studies.

Proposed Research:

During the first half of this project, we accomplished the first stated goal of our grant, which is to monitor gas fluxes over the surfaces of microbial mats *in situ*. During the second half of the granting period, we will focus on collecting more gas flux measurements at the site in Baja California, Mexico. We will also expand our data collection to other salt-flat environments that harbor microbial mats, such as those at Mono Lake, California. By monitoring gas flux at both sites, we will have a better understanding of the types and quantities of gases that evolve from different and geographically separated microbial communities. All of the data collected will accomplish the fourth stated goal of our grant, which is to model the impact of biogenic gas emissions on the atmosphere.

To accomplish the second stated goal of our grant (monitoring gas concentration as a function of depth in the mat), we will use voltametric micro-electrodes that are sensitive to O₂, H₂S, Fe(II), and Mn(II). Vertical profiles of oxidized and reduced regions of the mat, plus the cycling of iron and manganese, will show how different metabolisms occurring in each mat layer interact. Once we have a better understanding of how microbial mat communities behave over a diurnal cycle (both in the expulsion of gas and in the vertical cycling of nutrients), then we can begin the microbiological and molecular analysis of specific communities. Toward this end, samples will be collected, documented, and preserved for future work.

HIGHLIGHTS

Field Expeditions

Three field trips were made for our research: one in September 1999, one in November 1999, and one in February 2000, as discussed above. Research Assistants Lisa Stein, Ben Weiss, and Tanya Bosak accompanied me in these expeditions.

PUBLICATIONS & ABSTRACTS

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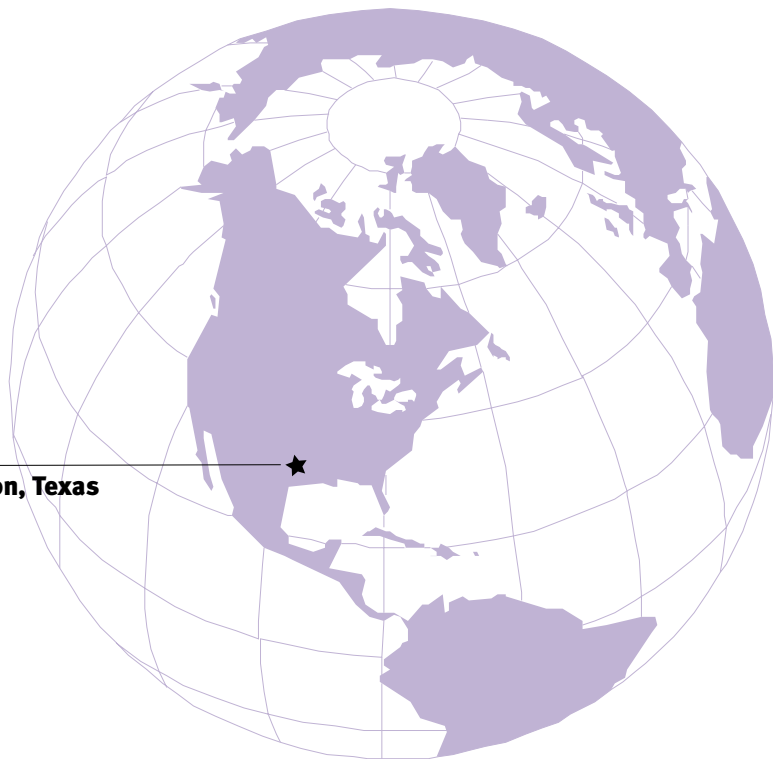
JOHNSON SPACE CENTER

**PRINCIPAL
INVESTIGATOR**



**David
McKay**

Houston, Texas



JOHNSON TEAM MEMBERS

Carlton Allen,
Johnson Space Center

Jaclyn Allen,
Johnson Space Center

Dennis Bazylinski,
Iowa State University

Mary Sue Bell,
Johnson Space Center

Nathalie Cabrol,
Ames Research Center

Henry Chafetz,
University Of Houston

Anita Dodson,
Johnson Space Center

George Flynn,
SUNY Plattsburgh

Everett Gibson,
Johnson Space Center

Sean Guidry,
University Of Houston

Larry Hersman,
Los Alamos National Laboratory

PROPOSAL EXECUTIVE SUMMARY (1998)

An important goal of the Astrobiology Institute is to determine how to recognize other biospheres and identify life beyond Earth. A critical aspect of this quest will be the analysis of rocks and related samples brought back from space. Finding undeniable evidence for current or past biologic activity in rock, soil, or water samples may be as simple as seeing live bugs moving about or finding obvious fossils. Indeed, for many terrestrial samples, this is the only evidence required. For other terrestrial samples, however, more is needed. Some Archean rocks are an example in which it is seldom obvious whether or not they once contained life. Very detailed study is generally needed. Thus far, the search for evidence of life in samples from space, done by analyzing various astromaterials (returned samples, meteorites, and cosmic dust), strongly indicates that such evidence may be subtle, uncertain, or subject to multiple interpretations. Three major examples are the search for evidence of past life in carbonaceous chondrites, in returned lunar samples, and in meteorites from Mars. All of these materials have been searched for signs of current or past life. Except for lunar samples, the results have been controversial, in part because of the lack of absolutely unequivocal biomarkers. Clearly, there is a need for new methods, new kinds of data, and new approaches to address this question to study meteorites and future returned samples from Mars, asteroids, and other solar system bodies. We must have a set of reliable and unassailable biomarkers. We must know how to search for them in samples and how to document either their presence or their absence. Overall, we must have this set ready by the time a Mars sample is brought back to Earth.

A good example of the search for life evidence in samples is provided by the study of Martian meteorite ALH84001. The lack of clear and obvious life forms or their fossils has made it difficult to show whether or not this meteorite contains certain signs of life. All of the evidence offered in support of this hypothesis remains circumstantial. Our original work on ALH84001 identified a number of features, which we proposed might be biomarkers (although we did not use that terminology). These features included the complex carbonates, the morphologic forms that resembled nanofossils and larger tubular fossils, the presence of polycyclic aromatic hydrocarbons (PAHs), plus the magnetite and the iron sulfides within the carbonates. We also commented on the close spatial association of these features. We stated that none of these features alone was diag-

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<http://www-sn.jsc.nasa.gov/astrobiology/biomarkers/index.html>

nostic of past life in the sample, but taken together they caused us to propose that hypothesis. We were essentially identifying a number of possible biomarkers. The reliability of each of these features as true biomarkers was questioned by a number of investigators (Jull et al. 1998, Bada et al. 1998, Bradley et al. 1997, Greenwood et al. 1997, Scott et al. 1997, Becker et al. 1997, Bradley et al. 1996, Shearer et al. 1996, Anders 1996). Nonbiologic origins were proposed for many of the features. However, we argued there were also problems with the proposed nonbiologic origins of the features, that the biologic origin of these features was still a viable hypothesis, and that, without more information, the question could not be firmly settled one way or the other. Using published literature with new analytical and experimental data, we propose to make a detailed study of selected biomarkers, document their properties, and construct a database for each. For each biomarker studied in detail, we will also study similar features made by nonbiologic processes, document these features and processes, and compare them to the true biomarkers. As with the biomarkers, we will search the literature, analyze natural systems, and conduct laboratory experiments.

A key objective of the proposed work is to identify and develop innovative new biomarkers (also termed "biological markers" or "biological signatures") not previously used on terrestrial rocks. Such new biomarkers will be documented, tried out on both modern and ancient rocks, and brought to a high state of readiness by the time Mars samples are returned. We will test these biomarkers, compare them to nonbiologic features from the literature or our own studies, and produce a detailed database summarizing the results. We propose to look for new biomarkers in each of these four major categories: biominerals; elemental and isotopic fractionation; morphologic features as seen at the micrometer and nanometer scale; and specific organic molecules. The goal is to develop a set of absolutely unequivocal biomarkers, along with methods to analyze for them, and to search for these biomarkers in astromaterials (including returned Mars samples). Along the way, we propose to learn much more about how organisms generate biomarkers and what such signatures tell us about the life cycle, evolution, ecology, and nature of the organisms and their interactions with the environment. In one sense, we will use biomarkers as clues to help us back track to important information about the organisms that left them. In turn, this information will contain clues to climate, atmosphere, and geologic processes through time.

Nancy Hinman,
University Of Montana

Richard Hoover,
Marshall Space Flight Center

Thomas Kieft,
New Mexico Institute of Mining and Technology

Marilyn Lindstrom,
Johnson Space Center

David McKay,
Johnson Space Center

Kitty Milliken,
University Of Texas, Austin

Penny Morris,
Johnson Space Center

James Papike,
University Of New Mexico

Lisa Prejean,
Johnson Space Center

Lisa Robbins,
USGS St. Petersburg

Chris Romanek,
University Of Georgia/SREL

Craig Schwandt,
Johnson Space Center

Andrew Steele,
Johnson Space Center

Kathie Thomas-Keprta,
Johnson Space Center

Allan Treiman,
Lunar And Planetary Institute

Norman Wainwright,
Marine Biological Laboratory

Maud Walsh,
Louisiana State University

Susan Wentworth,
Johnson Space Center

Frances Westall,
Johnson Space Center

We also propose to learn how totally nonbiologic processes can generate signatures that mimic biomarkers, producing biomarker impostors. A current example is the controversy over whether the carbonate globules in ALH84001 were generated by biologic or nonbiologic processes, and under what conditions. Ideally, we will learn to tell them apart, or, if this is not possible, we will use such information to advise extreme caution in applying these particular biomarkers. We will then narrow the list of true biomarkers to an absolutely unequivocal set. Along the way, we will learn much of how natural systems produce a variety of complex features by nonbiologic means. This knowledge will help us interpret the geologic history of astromaterials and their parent bodies.

To improve the prospects for swift and decisive determination of the question of life in returned Mars samples (as well as other astromaterials), we propose (as a member of the NAI) to start a long-term project to document currently-used biomarkers. Many of the currently-used biomarkers are similar to other features that can form by completely nonbiological processes. It may be difficult to differentiate between true biomarkers and these nonbiological impostors. We propose to document the nonbiologic impostors using techniques identical to those we use on the biomarkers and then closely compare them with each other. To accomplish this comparison, we propose to establish an innovative Green Team/Red Team organization. The Green Team identifies and documents biomarkers in various terrestrial analog examples, while the Red Team attempts to find these signatures produced in analog samples by totally nonbiological processes or to mimic these signatures in the laboratory under sterile conditions. The teams will use similar tools and instruments, produce comparable data, and make detailed comparisons of results to determine how closely the genuine biomarkers compare with the nonbiologic twin features or biomarker impostors. A unifying feature of our proposed work is that the Green Team and the Red Team will focus on the same sets of signatures, use many of the same analysis techniques, work closely together in a cooperative mode, and cross-check each other's ideas and results. This approach will ensure that multiple working hypotheses are carried throughout the investigations and that each biomarker is subject to very rigorous tests before it is certified as reliable. Some potential biomarkers may be eliminated because they prove impossible to distinguish from biomarker impostors, but a set of absolutely reliable biomarkers will hopefully emerge and be ready for scientists to apply to returned Mars samples as well as to other astromaterials. In either case, the hypotheses will be attached to the candidate biomarker in a comprehensive database available through the world.

We believe that a focused investigation of some existing and new biomarkers, along with their nonbiologic impostors, is absolutely vital to future space missions, which send back remote data and bring back samples from other planetary bodies. Only by having a set of reliable biomarkers will we be ready for the data and samples. The proposed JSC Institute for the Study of Biomarkers in Astromaterials will be a vehicle for investigating biomarkers and their nonbiologic impostors, as well as for developing a set of accepted and reliable markers for use by the entire scientific community.

Our team approach will be quite multidisciplinary. We have assembled from local scientists and outside partners an outstanding team of geologists, chemists, biologists, paleontologists, physicists, and astronomers, many of whom are already recognized as

leading experts in their respective fields, with some recognized internationally. We will take advantage of a large infrastructure already in place at JSC, where current research includes general petrologic and geochemical studies of astromaterials and their analogs. Additionally, we will work interactively with a number of advanced and specialized existing laboratories located at our partners' institutions. Overall, we will apply the same multidisciplinary approach to identifying and testing totally new biomarkers.

Coordination will be accomplished through weekly teleconferences, along with free exchange of information and data over the E-mail system and the Internet. Next Generation Internet will be used for: (1) two-way video communication; (2) exchange of data, images, and comment; (3) robotic field and sample collection exercises; and (4) remote operation of at least two instruments—the existing automated electron microprobe and a new FEG SEM (field emission gun, scanning electron microscopy), both at JSC. Team leaders will be responsible for coordinating the subtask activity of both their JSC team members and the partners at other organizations. An education and outreach leader will also participate in all the conferences and will establish a Web home page, which will be updated daily. We will have a major outreach program directed at teacher training, schools, and museums, both locally and at the partners' locations. We will assemble teacher exercises in astrobiology and try them with an existing core of senior teachers currently working with us on planetary science. Twice a year, the entire JSC team and outside partners will meet for two days to discuss results and strategy. Once a year, the JSC team and partners will meet with other members of the Astrobiology Institute at a California location for an Astrobiology Institute Conference. We will have an active program for summer training of teachers, summer undergraduate interns, year-round graduate students, and postdoctoral fellows.

JSC ASTROBIOLOGY INSTITUTE ACTIVITY OVERVIEW: YEAR 2

Biomarkers are specific properties of a rock, soil, or other sample that prove biogenic activity is taking place, or has taken place. Biomarkers include chemical, isotopic, spectral, and morphological signatures. As the number of independent biomarkers in a sample increases, so does the confidence that life is or was present.

The main goal of our Institute is to develop, characterize, and document a set of reliable biomarkers that can be used to identify present or past forms of microbial life. This work is divided into four ongoing projects, which are reported separately below:

- Biosignatures in Extraterrestrial Samples
- Biomarkers in Terrestrial Samples
- Biomarkers Database
- Astrobiology Education and Public Outreach

HIGHLIGHTS: YEAR 2

Flight Instrument Development

A proposal titled "Mars Immunoassay Life Detection Instrument" (David McKay, PI) was selected for funding by the NASA Office of Life and Microgravity Sciences and Applications and the Office of Space Flight Mars 2005 Definition Studies Program.

Investigators from JSC, the University of Portsmouth (UK), and Montana State University will develop a Mars lander instrument, using microchip immunoassay technology, to search for chemical biomarkers in the soil and rocks of Mars.

Mars Mission Science Team

Dr. Everett Gibson was selected as the only US member of the science team for the European Space Agency Beagle spacecraft. Beagle, the lander portion of the Mars Express spacecraft to be launched in 2003, will search for evidence at and below the surface of Mars.

New Research Staff

Dr. Simon Clemett joined our astrobiology group as an employee of Lockheed Martin Space Operations. Dr. Clemett recently completed a postdoctoral fellowship at Stanford, where he specialized in the analysis of trace organics using laser desorption mass spectrometry. He was a co-author on the original ALH84001 study, responsible for the analyses of polycyclic aromatic hydrocarbons. Dr. Clemett is establishing a laser desorption mass spectrometry lab within the JSC Astrobiology Program.

Dr. John Lisle joined our astrobiology group as an employee of Lockheed Martin Space Operations. Dr. Lisle is a microbial ecologist, specializing in microbial communities of Yellowstone hot springs and Antarctic dry valley lakes. He is also a Co-I on an upcoming space shuttle experiment to determine the effects of microgravity on bacteria. Dr. Lisle is establishing an extremophile research lab within the JSC Microbiology Program.

New Analytical Instruments

Our astrobiology group commenced operation of a new high resolution JEOL field-effect scanning electron microscope. The microbiology program commenced operation of a new Phillips environmental scanning electron microscope. These two instruments greatly enhance our ability to image and analyze living microorganisms, microfossils, and microscopic physical biomarkers.

Roadmap Objectives

#8

Past and Present Life
on Mars

Project

Biosignatures in Extraterrestrial Samples

Senior Project Investigator(s):
David McKay

OVERVIEW

This project concentrates on studying the meteorites in our systematic search for biosignatures in extraterrestrial samples. The Martian research by JSC and Co-I scientists currently includes four major biological / organic investigations, described below. In addition, we are conducting ongoing studies of chemical weathering on Mars and on Earth, as well as analyzing experimental shock effects to document the possibility of biomarker preservation in meteoritic materials. Finally, we have proposed to broaden the astrobiology investigation of these unique extraterrestrial samples through a new Inter-Institute

Focus Group on Martian Meteorites.

Analysis of Magnetite Crystals

Using transmission electron microscopy, we have analyzed magnetite crystals extracted from ALH84001 carbonate globules. Of the ALH84001 magnetites that we have examined, one population called elongated prismatic (~27% of the total) is indistinguishable from magnetites produced by certain strains of terrestrial magnetotactic bacteria. In addition, and just as important, there is no known inorganic population of magnetites that is analogous to the ALH84001 elongated prisms. Our interpretation is that the ALH84001 elongated prismatic magnetites were likely formed by biogenic processes on Mars.

Analysis of Features Morphologically Similar to Fossilized Terrestrial Organisms

Using high resolution scanning electron microscopy, we have continued our examination of features that are morphologically and chemically identical to known terrestrial mineralized/fossilized cells and/or cellular appendages. Our interpretation is that these features (present in the Martian meteorites ALH84001, Nakhla, and Shergotty) may have been formed by biological processes on Mars.

Detection of Trace-Level Organic Compounds

We have used Time-of-Flight Secondary Ion Mass Spectrometry to analyze specific features in ALH84001 and Nakhla for the presence of trace-level organic compounds. We have observed spatial relationships among some purported Martian fossilized cells and certain organic compounds. Future work on the organic analysis of known terrestrial biogenic features will help to determine if organic compounds found in the Martian meteorites may be interpreted as biomarkers.

Assessment of Terrestrial Microbial Contamination

We have used scanning electron microscopy and DNA/PCR (DNA/polymerized chain reaction) analyses to determine the extent of contamination in ALH84001 and Nakhla. It is clear that portions of all meteorites are contaminated with terrestrial materials (e.g., organic compounds, bacteria). If some of the fossilized biogenic-like forms in the meteorites are the mineralized remains of Martian organisms, then we must differentiate between indigenous Martian features and terrestrial contamination. This is possible only if we can constrain the extent and type of terrestrial contamination associated with each Martian meteorite. Our work shows that there are terrestrial bacteria and fungi living within and on the surfaces of ALH84001 and Nakhla. Our interpretation is that these terrestrial organisms are morphologically distinguishable from those we interpret as purported fossilized Martian cells or cellular appendages.

ACCOMPLISHMENTS

Progress on Year 2 Goals

- Investigation of possible microfossils and alteration products in Nakhla and Shergotty. Ongoing, with initial results presented at Lunar and Planetary Science Conference and Astrobiology Science Conference
- Documentation of occurrences of terrestrial biological contamination in Martian meteorites. Ongoing, with initial results presented at Lunar and Planetary Science Conference and Astrobiology Science Conference

Roadmap Objectives

#6

Microbial Ecology

#7

Extremes of Life

#17

Planetary Protection

Planned Work for Year 3

- Establish an Inter-Institute Mars Meteorite Focus Group
- Continue investigation of possible microfossils in Nakhla and Shergotty meteorites
- Continue to document chemical and physical weathering in Martian meteorites
- Continue analysis of shock effects in meteorites and analog materials

HIGHLIGHTS

- Biogenic Magnetite in ALH84001 (*Geochimica et Cosmochimica Acta*, in press)

This study determined a set of criteria that collectively comprise a biosignature based on terrestrial, intracellular magnetite. These criteria are so restrictive that no known population of inorganic magnetite has collectively displayed all these criteria. Furthermore, some populations of biogenic magnetite do not collectively meet all these properties. However, one population of magnetite crystals in Martian meteorite ALH84001 is identical to intracellular magnetite produced by terrestrial magnetotactic bacteria based on these criteria. Our interpretation is that the ALH84001 magnetites were formed by similar, biogenic processes on Mars.

- Weathering in Martian Meteorites

High-resolution electron microscope examination of Shergotty shows that it contains alteration products and secondary minerals very similar to those in Nakhla. Both meteorites were observed falls (Shergotty in India, 1865 and Nakhla in Egypt, 1911), so terrestrial weathering effects should be minimal. If present, they should also be very dissimilar. Both meteorites, however, contain clays and salt assemblages (NaCl, Ca sulfate, and Mg sulfate), which strongly suggest that the rocks were exposed to similar evaporitic near-surface environments on Mars. Evaporitic environments on Earth contain abundant microbes adapted to such conditions.

- Terrestrial Contamination in Martian Meteorites

One of our most important goals is to evaluate the extent of terrestrial contamination in extraterrestrial samples. This research has established that Martian meteorites, as well as many other carbon-containing meteorites, are rapidly contaminated by terrestrial fungi. We are now beginning to differentiate between these terrestrial contaminants and strikingly different, potentially biogenic, Martian features.

Project

Biomarkers in Terrestrial Samples

OVERVIEW

The importance of terrestrial samples to astrobiology cannot be overestimated. Only by

Year 2

understanding the evidence for life preserved in the rocks and soil of Earth can we assess indications of possible life elsewhere in the universe. We currently have four main thrusts of this research, described below.

Modern Life in Extreme Environments

We are studying samples from hot springs, caves, mines, and endolithic environments to document the presence of microbial life and its physical and chemical biomarkers.

Ancient Life

We are studying rocks that contain the earliest physical evidence of life on Earth, in order to document the retention of microbial forms and other biosignatures in the geologic record. Furthermore, we are investigating the variety of environments in which the most ancient life is preserved. This will hopefully aid interpretation of where and possibly how life started.

Natural Contamination of Fossiliferous and Non-Fossiliferous Lithologies

Fossiliferous rocks can become naturally contaminated through microbes living in cracks in both fossiliferous and non-fossiliferous lithologies, as well as through microbes living between grains in harsh environments. We are looking at fossilized microbes in cracks in Early Archaean, carbonaceous, fossiliferous cherts, as well as in serpentinized deep sea ultramafic rocks. Fossilized endolithic microorganisms in Arctic environments are also being investigated. Impact craters comprise another environment under investigation for fossilized endolithic organisms.

ACCOMPLISHMENTS

Experiments

We are conducting irradiation and shock experiments on terrestrial rocks to understand the effects of these stresses on samples from other planets. Some of the material being shocked is fossiliferous. Fossiliferous Early Archaean cherts are being experimentally metamorphosed to determine the effects of amphibolite/lower granulite metamorphism on the survival of carbonaceous microfossils. Films formed from prebiotic molecules are being artificially created as a comparison for biogenic films (biofilms) in order to distinguish differences between abiotic and biotic polymer films.

Progress on Year 2 Goals

- Survey of biomarkers in carbonate thermal springs. Complete, and manuscript accepted for publication in Icarus
- Complete survey of microbial fossils in Archean samples. Ongoing, with manuscript accepted for publication in Precambrian Research
- Biofilm research. Ongoing, with manuscript accepted for publication in Journal of Geophysical Research
- Bacteria fossilization experiments. Ongoing
- Studies of samples from submarine caves and deep mines. Ongoing, with initial

results presented at the Lunar and Planetary Science Conference

Planned Work for Year 3

- Document the interactions of microorganisms with iron oxides in Archaean chert and banded iron formations
- Determine the effects of high shock pressures on minerals found in Martian meteorites
- Determine the mechanism and effect of microbial biofilm preservation in the geologic record

HIGHLIGHTS

- Physical Biomarkers in Carbonate Hot Springs (*Icarus*, in press)

Physical evidence of life (physical biomarkers) from the deposits of carbonate hot springs were documented at the scale of microorganisms – submillimeter to submicrometer. The four moderate-temperature (57°C to 72°C) and neutral pH springs in this study support diverse communities of bacteria adapted to specific physical and chemical conditions. Some of the microbes coexist with travertine deposits in endolithic communities. In other cases, the microbes are rapidly coated and destroyed by precipitates, but they leave distinctive mineral fabrics. Some microbes adapted to carbonate hot springs produce extracellular polymeric substance, which forms a three-dimensional matrix with living cells and cell remains, known as a biofilm. Silicon and iron oxides often coat the biofilm, leading to long-term preservation. Submicrometer mineralized spheres composed of calcium fluoride or silica are common in carbonate hot spring deposits. Sphere formation is biologically mediated, but the spheres themselves are apparently not fossils or microbes. Additionally, some microbes selectively weather mineral surfaces in distinctive patterns. Hot spring deposits have been cited as prime locations for exobiological exploration of Mars. The presence of preserved microscopic physical biomarkers at all four sites supports a strategy of searching for evidence of life in hot spring deposits on Mars.

- Early Archaean Fossil Bacteria and Biofilms in Hydrothermally-Influenced Sediments from the Barberton Greenstone Belt, South Africa (*Precambrian Research*, in press)
SEM (scanning electron microscopy) imaging of HF (hydrofluoric acid) etched, 3.3-3.5 Ga cherts from the Onverwacht Group (South Africa) reveals small spherical (1 mm diameter) and rod-shaped structures (2-3.8 mm in length), which are interpreted as possible fossil coccoid and bacillar bacteria (prokaryotes), respectively, preserved by mineral replacement. Other possibly biogenic structures include smaller rod-shaped bacteriomorphs (<2 mm in length) and bacteriomorph molds. The identification of these structures as fossil bacteria is based on size, shape, cell division, distribution in colonies and occurrence in biolaminated sediments. The exceptionally fine conservation has preserved textures such as wrinkled outer walls on the coccoid fossils, while the bacillar fossils are turgid. Carbon isotope analyses support the presence of bacteria in these cherts with $\delta^{13}\text{C}$ values around – 27 per mil. The cherts are characterized by fine, wavy laminae created by granular to smooth or ropy-textured films coating bedding planes, interpreted as probable bacterial biofilms, which have been pseudomorphed by minerals. Although most of the Onverwacht Group was deposited in relatively deep water (>900 m), textures in the sediments in which these biogenic structures occur suggest that

they were probably deposited in a shallow water environment subjected to intermittent subaerial exposure. Pervasive hydrothermal activity is evidenced by oxygen isotope studies as well as by the penecontemporaneous silicification of all rock types by low temperature ($\sim 22^{\circ}\text{C}$) hydrothermal solutions.

- The Nature of Fossil bacteria: A Guide to the Search for Extraterrestrial Life (*Journal of Geophysical Research*, 07/99)

In an attempt to establish reliable criteria for identification of potential fossil life in extraterrestrial materials, the fossilizable characteristics of bacteria (namely, size, shape, cell wall texture, association, and colony formation) are described. In addition, an overview is given on various ways in which fossil bacteria are preserved: as compressions in fine-grained sediments; preservation in amber; permineralized by silica; replacement by minerals such as silica, pyrite, Fe/Mn oxides, calcite, phosphate, and siderite; or as molds in minerals. The problem of confounding minerally replaced bacteria with nonbiological structures having a bacterial morphology is addressed. Examples of fossilized bacteria from the Early Archaean through to the Recent are used to illustrate the various modes of preservation and the morphology of fossil bacteria.

- Biofilms as Biomarkers in Terrestrial and Extraterrestrial Materials (*Journal of Geophysical Research*, in press)

Organic polymeric substances are a fundamental component of microbial biofilms. Microorganisms, especially bacteria, secrete extracellular polymeric substances (EPS) to form slime layers in which they reproduce. In the sedimentary environment, biofilms commonly contain products of bacterial degradation as well as autochthonous and allochthonous mineral components. They are complex structures that serve as protection for the colonies of microorganisms living in them and also act as nutrient traps. Biofilms are almost ubiquitous wherever there is an interface and moisture (liquid/liquid, liquid/solid, liquid/gas, solid/gas). In sedimentary rocks, they are commonly recognized as stromatolites. The EPS and cell components of the microbial biofilms contain many cation chelation sites, which are implicated in the mineralization of the films. EPS, biofilms, and their related components, thus, have strong preservation potential in the rock record. Fossilized microbial polymeric substances (FPS) and biofilms appear to retain the same morphological characteristics as the unfossilized material and have been recognized in rock formations dating back to Early Archaean (3.5 b.y.). We describe FPS and biofilms from hot spring, deep sea, volcanic lake, and shallow marine/littoral environments ranging up to 3.5 b.y. in age. FPS and biofilms are more common than fossil bacteria themselves, especially in the older part of the terrestrial record. The widespread distribution of microbial biofilms and their great survival potential makes their fossilized remains a useful biomarker as a proxy for life with obvious application to the search for life in extraterrestrial materials.

- Gamma Sterilization of Mars Rocks and Minerals (*Journal of Geophysical Research*, 11/99)

Rock and soil samples from the planet Mars are due to be returned to Earth within a decade. Martian samples initially will be tested for evidence of life and biological hazard under strict biological containment. Wider distribution of samples for organic and inorganic analysis may occur only if neither evidence of life nor hazard is detected, or if the samples are first sterilized. We subjected a range of Mars analog rocks and min-

erals to high doses of gamma radiation in order to determine the effects of gamma sterilization on the isotopic, chemical, and physical properties of the samples. Gamma photons from ^{60}Co (1.17 and 1.33 MeV) in doses as high as 3×10^7 rads did not induce radioactivity in the samples and produced no measurable changes in their isotopic and chemical compositions. This level of irradiation also produced no measurable changes in the crystallographic structure of any sample, the surface areas of soil analogs, or the fluid inclusion homogenization temperature of quartz. The only detectable effects of irradiation were dose-dependent changes in the visible and near-infrared spectral region (e.g., discoloration and darkening of quartz and halite and an increase in albedo of carbonates) and increases in the thermoluminescence of quartz and plagioclase. If samples returned from Mars require biological sterilization, gamma irradiation provides a feasible option.

Field Expeditions

Barberton Greenstone Belt, South Africa

Frances Westall (JSC / Lunar and Planetary Institute) conducted studies of microfossils and their environments in the Early Archaean Barberton greenstone belt. Within the framework of searching for the earliest signs of life on Earth, this project seeks to study the variety of microbial fossils within Early Archaean cherts. Additionally, information concerning the variety of environments in which the fossils are found is being collected. Thin section and electron microscope methods are used.

Villa Luz Cave, Mexico

Carlton Allen (Lockheed Martin / JSC) joined a team of 18 biologists, geologists, and cavers (all led by Dr. Louise Hose of Westminster College) for a January 2000 expedition to Villa Luz. This is one of the few caves in the world being formed by sulfuric acid dissolution from H_2S -rich springs. The cave supports a rich ecosystem based on sulfur-oxidizing acid-tolerant bacteria as well as spiders, midges, fish, and bats. Dr. Allen's work is concentrated on mineral biomarkers, notably large-scale bacterially-mediated precipitation of pyrite (FeS_2).

Yellowstone National Park, WY

Sean Guidry and Yongqiang Wu (University of Houston) spent two weeks at Yellowstone as part of Guidry's dissertation research. They collected microorganisms and biomarkers preserved in silica and carbonate hot spring deposits in the Geyser Basin and the Mammoth complex. This investigation is evaluating the preservation potential of microbial organisms at hot spring deposits. The study involves collection of material actively growing and being mineralized at the hot spring surface and comparing its preservation with successively more deeply buried (older) and diagenetically altered material.

Mormon Mesa, Mesquite, NV

Allan Treiman joined colleagues from the Lunar and Planetary Institute and the Aerospace corporation for a field and remote sensing study of the Mormon Mesa caliche hardpan in December, 1999. The hardpan is an areally extensive carbonate-rich soil, with significant evidence for biological activity. The field work included acquisition of infrared spectra, both in reflectance and in thermal emittance.

Sacramento Mountains, NM

Yongqiang Wu and Sean Guidry (University of Houston) spent 12 days in the field as

Year 2

part of Wu's Ph.D. dissertation research. It has been proposed that carbonate mud (micrite) comprising mud-mounds, in general, are bacterially induced precipitates. This investigation is specifically to determine: (1) if the micrite comprising the mud-mounds within the Mississippian Sacramento Limestone is bacterially induced precipitate; and (2) if so, what biomarkers can be found in this 300 million year old deposit.

Biomarkers Database

Project

OVERVIEW

The JSC Astrobiology group's main goal is to develop, characterize, and document a set of reliable biomarkers that can be used to identify present or past forms of microbial life. The research efforts of many groups are being consolidated in a continuously-updated electronic database.

ACCOMPLISHMENTS

Progress on Year 2 Goals

- Comprehensive, interactive database of biomarkers and false positives. Framework complete, with review and input in progress

Planned Work for Year 3

- Complete a web-based comprehensive, interactive database of biomarkers and false positives

HIGHLIGHTS

Biomarkers Database on the Web

The Biomarkers Database was designed, reviewed, and placed on the JSC Astrobiology web page for input. This is the specific website: <http://sn-io.jsc.nasa.gov/sn/astrobiology/biomarkers/index.html>

Senior Project Investigator(s):

David McKay

Roadmap Objectives

#6

Microbial Ecology

#7

Extremes of Life

PUBLICATIONS & ABSTRACTS

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PROJECT TITLE: *Biomarkers in Terrestrial Samples*

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PROJECT TITLE: Mars Meteorite Focus Group (Pending)

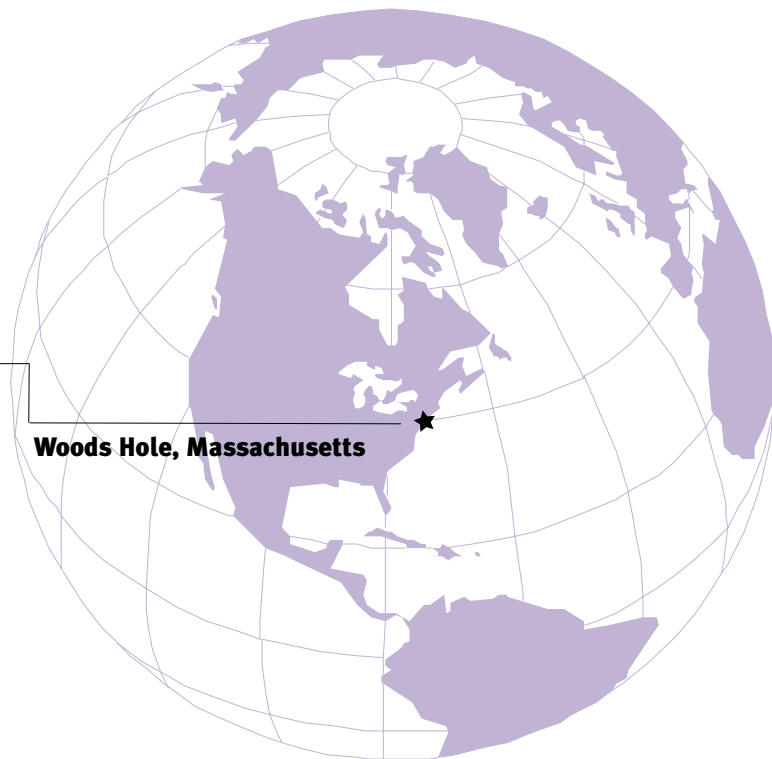
Year 2

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MARINE BIOLOGICAL LABORATORY



Woods Hole, Massachusetts

PRINCIPAL INVESTIGATOR



Mitchell
Sogin

MBL TEAM MEMBERS

Linda Amaral Zettler,
Marine Biological Laboratory

Ricardo Amils,
Centro de Astrobiología

Mike Atkins,
Woods Hole Oceanographic Institute

David Beaudoin,
Marine Biological Laboratory

David Caron,
University of Southern California

Michael Cummings,
Marine Biological Laboratory

Alvin de Vera Gomez,
Woods Hole Oceanographic Institute

Mark Dennett,
Woods Hole Oceanographic Institute

Virginia Edgcomb,
Marine Biological Laboratory

Rebecca Gast,
Woods Hole Oceanographic Institute

Felipe Gomez,
Centro de Astrobiología

PROPOSAL EXECUTIVE SUMMARY (1998)

The planet Earth is about 4.7 billion years old. Our first living forms on Earth appeared 3.5-4.2 billion years ago, and they consisted of microscopic, relatively simple cells (Schopf, 1983). Over the ensuing billions of years, primitive cells evolved into the ten million or more different species that represent the existing biological diversity on our planet. All organisms (including animals, plants, fungi, and an untold collection of microbial species) have their common ancestral roots within these earliest life forms. Insights into historical events that dominated the evolution of this biosphere are linked to understanding phylogenetic relationships between the major groups of extant organisms and elucidating processes that explain their evolutionary history.

An important goal of the NASA Astrobiology Institute is to gain information about the phenotypic evolution of early life forms and to elucidate how changing environments contributed to the development of complex systems in simple organisms. From this understanding, we will be better prepared for future discoveries about extraterrestrial life that might come from sample return missions or exploration of other planets.

An important concern about planetary exploration and sample return missions is the possibility of biological contamination of Earth's biosphere with extraterrestrial organisms. The greatest problem is the possibility of contamination from microscopic extraterrestrial life forms. These organisms are more difficult to detect than multicellular taxa. Also, their capacity to survive extreme conditions makes them the most likely to survive on small solar system bodies or otherwise uninhabitable planetary bodies. Any assessment of biological risk associated with extraterrestrial exploration must be based upon what we know about microbial diversity of the organisms for which we have physiological information. Yet, by some estimates only 1% of the microbial forms on Earth have ever been cultured or described. Therefore, we are almost completely ignorant of the vast majority of microbial life, and there is almost no information about how they evolve in response to environmental change. Furthermore, our current life detection technologies are probably inadequate for planetary exploration. At a minimum, we should develop a more thorough understanding of minimal constraints for the possible origins and evolution of even the most primitive microbes.

Year 2

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Certainly, the first self-replicating terrestrial organisms had simple, prokaryotic-like architecture. The evolution of more complex organisms (cellular forms with a nucleus) required the invention of new molecular features such as cytoskeletal proteins and new membrane structures. Given the available data, we cannot identify the most important early molecular innovations. Therefore, we know little about how the eukaryotic cell was first assembled. We also have little information about the processes underlying major changes in eukaryotic genomes. For example, endosymbiosis was and may continue to shape how eukaryotic genomes evolve, but we lack molecular details about how this important process occurs.

Our NAI proposal has three major themes united by common scientific interests and resource requirements for studies of molecular evolution:

- Patterns and Mechanisms of Evolutionary Diversity in Microbial Populations
- Evolution of Complex Systems in Simple Animals
- Evolution of Phenotype, Genotype and Microbial Genomes

Patterns and Mechanisms of Evolutionary Diversity in Microbial Populations

The first theme will bring together environmental microbiologists and molecular evolutionists with the common goal of elucidating the prokaryotic and eukaryotic microbial diversity in selected thermophilic and mesophilic environments. We will also propose a set of experiments to address the molecular basis of endosymbioses. Endosymbionts have the potential of dramatically expanding the genetic repertoire of eukaryotic genomes. These investigations will serve as a platform for extensive interactions and sharing of resources between members of the Josephine Bay Paul Center for Comparative Molecular Biology and Evolution at the Marine Biological Laboratory and microbial ecologists in the Biology Department of the Woods Hole Oceanographic Institution. To achieve their scientific goals, these investigators will exchange samples from geographically-diverse sites, and they will be processed in a common-use facility for extracting nucleic acids.

Katherine Hammar,
Marine Biological Laboratory

Holger Jannasch,
Woods Hole Oceanographic Institute

Brendan Keenan,
Marine Biological Laboratory

Alastair Kerr,
Marine Biological Laboratory

David Kysela,
Marine Biological Laboratory

Ping Liang,
Marine Biological Laboratory

Andrew McArthur,
Marine Biological Laboratory

Laura McNerney,
Marine Biological Laboratory

Dawn Moran,
Woods Hole Oceanographic Institute

Lorraine Olendzenski,
Marine Biological Laboratory

David Patterson,
University of Sydney

Monica Riley,
Marine Biological Laboratory

Andrew Roger,
Dalhousie University

Alastair Simpson,
University of Sydney

Peter Smith,
Marine Biological Laboratory

Mitchell Sogin,
Marine Biological Laboratory

Andreas Teske,
Woods Hole Oceanographic Institute

Evolution of Complex Systems in Simple Animals

The second theme will take advantage of molecular expertise in the Josephine Bay Paul Center and the state of the art Marine Resources Center's capacity to secure and maintain diverse marine organisms. One of these projects will "phylogenetically screen" for coding regions that play crucial roles in the renin-angiotensin system. The complete renin-angiotensin system has only been found in vertebrates, but there is evidence for each of its components in separate invertebrate taxa. The central question is to determine at what point did a complete renin-angiotensin system evolve in animal phyla during this biosphere's evolutionary history. From an evolutionary perspective, renin-angiotensin or related endocrine functions likely played an important role as animals moved from buoyant marine environments to fresh water and eventually to land. The renin-angiotensin system is one of the most potent vasal constrictors and hence plays an important role in regulating fluid pressure. This system is also very important from the perspective of space flight because it plays a major role in fluid retention. Understanding how it evolved may provide clues about underlying mechanisms. Study of it is likely to identify superior model systems for functional analyses.

A second project within the above evolution theme is to study evolution of host defense mechanisms in simple invertebrates. The described experimental system is for horseshoe crabs, which like other invertebrates must resist bacterial, protistan, and fungal predators. We recognize that horseshoe crabs are not exactly simple. However, they will provide a basis for further phylogenetic studies in earlier diverging animal phyla such as cnidaria or even within the world of protists. Both of these projects employ similar molecular methodologies. They also offer excellent examples of how molecular evolution can be used to study evolution of complex systems in response to environmental change.

Evolution of Phenotype, Genotype and Microbial Genomes

The final theme has two major projects. The first is a microbial experimental model system that will explore drug resistance changes, in response both to altered environments as well as to an invertebrate system for G-coupled protein evolution in the eyes of animals and certain other cell surface receptors. The second project is a molecular evolution study of proteins that were duplicated in ancient genomes. It will take advantage of the rapidly growing data base of genome sequences from divergent bacteria. Both projects will provide insights about how changes in the environment can affect genome evolution. For example, light is an important environmental factor that impacts most life forms. Clearly, the evolution of receptors such as cephalopod rhodopsin is important for understanding how complex systems evolved to take advantage of interpreting light emission patterns. Genome analyses also have potential for finding correlations between the very different environments preferred by different organisms (particularly the Archaea) and the kinds and groupings of proteins in those organisms.

The Woods Hole scientific community has a strong commitment to studies related to astrobiology. Five of the co-investigators on this proposal (Dr. Sogin, Dr. Caron, Dr. Gast, Dr. Jannasch, and Dr. Teske) are currently funded through the LExEn program for studies of other microbial environments. Dr. Sogin has an active research and workshop program funded through the Exobiology program, NIH, and NSF. This proposal is designed to integrate into and complement the existing LExEn and Exobiology projects

Year 2

through use of shared technologies and scientific expertise.

This NAI proposal includes a strong educational and outreach program. (This program was fully described in the TRAINING PLAN AND EDUCATION/PUBLIC OUTREACH PLAN section of the MBL Proposal for NAI funding.) The Marine Biological Laboratory (MBL) has a strong commitment to education and training of established investigators, post-doctoral students, graduate students, undergraduates, and high school teachers. This NAI proposal plans to help support courses and fellowships in evolutionary developmental biology, molecular evolution, teacher enhancement, and the education of professional science writers. All of these programs are ongoing and available to participants by competitive application. We will also maintain a website to describe activities and research accomplishments in our astrobiology program as well as to provide access to information presented in lectures. The website will contain related content information, instructions for hands-on activities, and links to already existing sites with related educational materials. As part of our Astrobiology efforts, we will take advantage of high speed network connections for analysis of research data and for video conferencing, all related to education and outreach. We have formally agreed to participate with the Harvard-MIT Astrobiology Consortium in a broad based education program. In addition to faculty exchange visits between the two research communities, we will use the high speed network for presenting lectures to remote audiences. With high speed networking, it will be possible to make more efficient use of our computational facilities and to take advantage of spare CPU (central processor unit) cycles on remote machines.

The institutional commitment includes dedicated laboratory space, capital equipment for laboratory research, and facilities for theoretical analyses. This commitment is backed by nearly \$856,429 in cost-sharing spread out over a five year period. Furthermore, at least one additional faculty position will be filled this year in the Josephine Bay Paul Center for Comparative Molecular Biology and Evolution in a discipline that will be highly relevant to this astrobiology proposal. It is inappropriate at this time to assign a cost-share value to that commitment.

Both the Marine Biological Laboratory and the Woods Hole Oceanographic Institution are committed to full participation in the virtual NASA Astrobiology Institute. If funded, we intend to cooperate fully with other programs in astrobiology in terms of sharing resources and new information gained from the proposed investigations.

Protist Diversity in Extreme Environments

Project

Senior Project Investigator(s):
D.A. Caron and R.J. Gast

ACCOMPLISHMENTS

In the past year, we have continued to test and refine methods for examining the protist populations from natural environments. Our full-length small subunit ribosomal gene libraries are now screened using group-specific oligonucleotide (oligos) probes

Roadmap Objectives

#6
Microbial Ecology

#7
Extremes of Life

that we have designed for diatoms and dinoflagellates. We have also designed oligos to several other groups (such as the Chrysophytes, the Pelagophytes, and the Bodonids), and we are in the process of testing them. Most of the methods were developed in collaboration with our LExEn program, which is directed toward the study of protistan diversity in Antarctic environments. These methods are now being applied to several types of samples from different (and somewhat extreme) environments.

We collected anaerobic sediment samples from a tidal creek (Trunk River) in Cape Cod (Massachusetts) in late September of 1999. Water samples from the strongly sulfidic layers of Blue Holes on Andros Island were also collected. (These layers can occur anywhere from 20 to 70 feet below the surface, with temperatures around 25°C and sulfide concentrations ranging from 10 micromolar to 10 millimolar.) Preliminary sequence analysis of the Trunk River samples has identified ciliates, apicomplexans and an acantharian. (This is very surprising, and we are pursuing this sequence in order to identify whether it is real or something only related to acantharia).

During the next year, we will generate full-length ribosomal clone libraries and screen them using probes made from DGGE (denaturing gradient gel electrophoresis) gel bands, in order to correlate the bands with full-length sequences, rather than sequence all of them first. This will help us in our work this summer, 2000. We will use DGGE to monitor the protistan community changes in Trunk River as the environment becomes anaerobic. (See Field Expeditions section below.) For the Andros samples, we will attempt to recover protistan cultures from the samples, as well as extract and analyze DNA from the original samples. We will do this to examine the protistan populations and determine whether deep/early branching eukaryotes can be identified from these environments.

HIGHLIGHTS

Field Expeditions

Summer (2000), Trunk River, Cape Cod, Massachusetts

We (David Beaudoin, Mark Dennett, Dawn Moran, Dave Caron, and Rebecca Gast) will again collect and analyze samples from Trunk River prior to, and during, its anaerobic events. In addition to monitoring the genetic changes, we will be trying to establish cultures of anaerobic protists for future studies of their basic physiology and growth rate.

Roadmap Objectives

#4

Genomic Clues to Evolution

Project

Relationship of Genetic Changes to Phenotypic Changes in Organism - Environment Interactions

Senior Project Investigator(s):
M. Cummings

ACCOMPLISHMENTS

The principal goal of this research is to understand and model genotype-phenotype relationships through studies of variation in opsin genes from closely related species. While

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this goal has been maintained, the research design has changed to eliminate all work on drug resistance in *Mycobacterium tuberculosis*, which is obviously more of medical interest. Instead, we will focus entirely on the model system of opsins and light absorption. Also, the research organisms for this work have been changed from Cephalopoda (squid, octopi) to Odonata (damselflies and dragonflies).

For the work on opsins, Odonata offer substantial advantages:

1. Ease of collecting: Odonates are easily collected using insect nets.
2. Local species diversity: Approximately 105 species of odonates occur on Cape Cod.
3. Gene diversity: Odonates have 4-6 opsin genes per species, which greatly increases the sampling effectiveness.

Together, these advantages have already provided a greatly improved sampling efficiency and species diversity. They will also result in greater genotype and phenotype diversity, thus increasing the power of the study. The research started with field collection of 26 species of dragonflies and damselflies. These species represent six families and fifteen genera, providing a broad range of diversity within the group.

We have prepared poly(A)-RNA from all 26 species. Using RT-PCR (reverse transcriptase-polymerase chain reaction), we prepared complementary DNA (cDNA) for most of these samples, and they were subsequently cloned. We have sequenced at least two opsin cDNA clones from each of 16 species, and we have collected single sequences from most of the other field samples.

Planned work for the next year include the following: (1) a week-long intensive field collecting trip to Maine, during which time we expect to double the number of species in the study; (2) continuing laboratory work with an emphasis on increasing the efficiency of the sequencing of the cDNA clones; (3) building a bioinformatics infrastructure to increase the automation of data processing using Perl and other tools; and (4) building a project database using MySQL and PerlDBI.

HIGHLIGHTS

Field Expeditions

1. Massachusetts, July-September 1999. Michael P. Cummings, Laura A. McInerney and volunteers were collecting specimens.
2. California, April 2000. Michael P. Cummings was collecting specimens.
3. Florida, May 2000. Laura A. McInerney was collecting specimens.

Eukaryote Origins and the Evolution of Cellular Complexity – Evolution of Tubulins

Project

Senior Project Investigator(s):

V.P. Edgcomb, M.L. Sogin, D.J. Patterson, and A.J. Roger

ACCOMPLISHMENTS

Jakobids are free-living, heterotrophic flagellates that might represent early diverging,

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mitochondriate protists. They share ultrastructural similarities with eukaryotes that occupy basal positions in molecular phylogenies. Their mitochondrial genome architecture is also eubacteria-like, suggesting a close affinity with the ancestral alpha-proteobacterial symbiont that gave rise to mitochondria and hydrogenosomes.

To elucidate relationships among jakobids and other early-diverging eukaryotic lineages, we have characterized alpha- and beta-tubulin genes from four jakobids: *Jakoba libera*, *Jakoba incarcerata*, *Reclinomonas americana* (the "core jakobids"), and *Malawimonas jakobiformis*. These are the first reports of nuclear genes from these organisms. Phylogenies based on alpha-, beta-, and combined alpha- plus beta-tubulin protein data sets do not support the monophyly of the jakobids. Beta-tubulin and combined alpha- plus beta-tubulin phylogenies show a sister-group relationship between *J. libera* and *R. americana*. However, the two other jakobids, *M. jakobiformis* and *J. incarcerata* have unclear affinities. In all three analyses, *J. libera*, *R. americana* and *M. jakobiformis* emerge from within a well-supported large "plant-protist" clade that includes plants, green algae, cryptophytes, stramenopiles, alveolates, Euglenozoa, Heterolobosea, and several other protist groups. However, animals, fungi, microsporidia, parabasalids, and diplomonads are not included in this affinity relationship.

A preferred branching order within the plant-protist clade is not identified, but there is a tendency for the *J. libera*-*R. americana* lineage to group with a clade made up of the heteroloboseid amoeboflagellates and euglenozoan protists. *Jakoba incarcerata* branches within the plant-protist clade in beta- and alpha- plus beta-tubulin phylogenies. In alpha-tubulin trees, *J. incarcerata* occupies an unresolved position. It groups weakly with the animal/fungal/microsporidian group or with amitochondriate parabasalid and diplomonad lineages, depending on the phylogenetic method employed. Tubulin gene phylogenies are in general agreement with mitochondrial gene phylogenies and ultrastructural data, indicating that the "jakobids" may be polyphyletic. Relationships to the putatively deep-branching amitochondriate diplomonads remain uncertain.

HIGHLIGHTS

- Work described here presents strong evidence that the excavate hypothesis for the origins of eukaryotes is not likely to be correct.
- This work also convinces us that studies of tubulin will not be of major importance in identifying early branching patterns in the eukaryotic line of descent.

Project

Diversity of Eukaryotes in Thermophilic and Mesophilic Environments That Might Resemble Early Earth's Biosphere

Senior Project Investigator(s):

V. Edgcomb, M.L. Sogin, and A. Teske

ACCOMPLISHMENTS

Guaymas Basin Molecular Survey of Eukaryotic Communities.

Environmental conditions in anaerobic marine sediments may closely resemble those under which eukaryotes diverged from a prokaryote precursor 2-3 billion years ago. Yet little is known about eukaryotic microbial populations in these environments, thus leaving a substantial gap in our understanding of vent biota. We have initiated a study of eukaryotic microbial community composition based upon SSU rDNA (small subunit ribosomal DNA) sequence analyses of sediments from Guaymas Basin (Gulf of California). Temperature profiles were measured concurrently at 5-cm depth intervals, and the cores were sectioned anaerobically into 1-cm segments. We used eukaryotic-specific primers with PCR (polymerase chain reaction) to amplify rDNA genes. Sequences of the amplified products were merged with a large data base of eukaryotic ribosomal RNAs and subjected to phylogenetic analyses.

PCR amplifications were achieved for samples from 0-3 cm (60°C) depth in core A and from 0-3 cm (40°C) plus a seawater interface sample in core C. We have sequenced ~45 clones from each of the 1-cm horizons from both cores. Phylogenetic analysis of horizons 0-1 from core C and 0-1 and 1-2 from core A reveal a broad array of eukaryote taxa. Many sequences cluster among known taxa: metazoa, green plants, dinoflagellates, apicomplexa, ciliates, stramenopiles (e.g., diatoms), and fungi. Several sequences could not be assigned to any known eukaryotic phyla. Several closely related clones from the 0-1 and 2-3 cm horizons of core C were found to branch deep within the eukaryote lineage, between the parabasalids and the eukaryotic crown taxa. Of particular interest among the cloned rDNAs is the deep-branching clone C1_E027. Its position between described anaerobic, early eukaryotes is evidence of a primitive, previously undescribed organism. Although this sequence was obtained from a relatively cool (3-25°C) sediment sample, the spatially and thermally dynamic nature of hydrothermal vents imply that the organism might reside at even higher temperatures. We have already demonstrated a remarkable diversity of eukaryote rDNAs in hydrothermal vent sediments, but we have only scratched the surface of the potential diversity in this environment.

HIGHLIGHTS

- This work is the first description of eukaryotic microbial diversity in a hydrothermal vent community. It reveals the existence of diverse eukaryotes, including lineages that may be basal to most if not all other eukaryotes.
- We anticipate that studies of warmer regions of these cores will dramatically advance our knowledge about the diversity of protists and may lead to exciting discoveries about the range of conditions under which eukaryotes can survive.

Field Expeditions

Field work for this study was carried out using the deep-sea submersible, Alvin. The research team included international collaborators who carried out a multidisciplinary study of the core samples. In addition to research on microbial populations, various conditions (temperature profiles, density measurements, lipid analyses, pore water chemistry, etc.) were also studied. All of the data will be correlated in order to provide a detailed description of this unusual hydrothermal environment.

Roadmap Objectives

#2

Origin of Life's Cellular Components

Cross Team Collaborations

As is true for our studies of prokaryote diversity in Guaymas, results of these studies were discussed with members of other astrobiology teams at the *First Science Conference on Astrobiology* (April 2000), held at Ames Research Center. No formal collaborations have developed, but there are obvious potential ties with the Carnegie Institution of Washington group.

Project

Genes That Regulate Photosymbiotic Relationships

Senior Project Investigator(s):

R.J. Gast and D.A. Caron

ACCOMPLISHMENTS

Work on the isolation of symbiosis-specific genes from algal-sarcodine photosymbiotic relationships has begun with the utilization of cultures of free-living symbionts that we have in the laboratory. During the past year, we have optimized several kits and methods from Clontech for the generation of cDNA (complementary DNA) from mRNA (messenger RNA) and suppression-subtractive hybridization. We have used RNA isolated from two different dinoflagellate symbionts in their free-living states to test the methods. cDNA libraries were generated for both, and we have successfully performed suppression-subtractive hybridization utilizing them.

Our next step is the collection of intact symbioses, and we are currently planning two field sessions for the collection of the sarcodines in June and in August. (See Field Expeditions section below.) We have been using RNA isolation methods that enable us to extract nucleic acids from a small number of cells (about 10,000). Therefore, we have decided to utilize intact symbioses rather than continue to try to refine the inducible system that we originally proposed. Symbionts will be microdissected from their hosts immediately upon collection, and the symbionts from at least 20 of the same host will be pooled for extraction. This should provide us with at least 20,000 symbiont cells for RNA collection. We will use the cDNAs from the free-living symbionts to perform the suppression-subtraction hybridization, then we will begin our identification and analysis of the differentially expressed genes.

Although this project has been slow in developing, we have made a significant step forward in our progress this year with the free-living symbionts. We are poised to make the next step during 2000-2001. Our work will focus largely upon recovering and identifying genes expressed in the symbiotic state of the algal symbionts that we are studying. The initial analyses will utilize the natural symbionts of the host organism, *Thalassicolla nucleata*. Once this has been accomplished, we will begin to work with some of the foraminiferan hosts and their symbionts.

HIGHLIGHTS

Field Expeditions

Our first field expedition will take place June 8-9 (2000) onboard the *Weatherbird II*. Dr.

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Gast will collect planktonic sarcodines in the Sargasso Sea, south of Bermuda. This cruise is for the Bermuda-Atlantic Time Series studies conducted at the Bermuda Biological Station for Research, and we are included as ancillary personnel.

Our second expedition will take place August 17-31 (2000) onboard the *Endeavor*. Dr. Gast will be collecting planktonic sarcodines at currently undecided locations in the Atlantic Ocean south of Bermuda. This is once again an ancillary project, as the cruise is being supported by an NSF Biological Oceanography grant to David Caron, Rebecca Gast and Mark Dennett.

Both of the above field excursions will be used to collect intact host-symbiont associations for the isolation and analysis of symbiotically expressed RNAs.

Ancestry of the Earliest Proteins

Project

Senior Project Investigator(s):
Monica Riley

ACCOMPLISHMENTS

The focus of our work is reconstruction of our evolutionary beginnings by characterizing the very earliest ancestral proteins that existed even before the theoretical Last Common Ancestor cell. By analyzing the sequences of extant proteins and grouping them by commonalities, we should be able to reconstruct the numbers and types of earliest ancestor molecules of life on Earth.

We have identified a very large family of weakly-similar proteins, which could have arisen from a single ancestor that generated these four types of enzymes: dehydrogenases, epimerases, isomerases and dehydratases. Both PSI-BLAST and DARWIN sequence analysis protocols were used to examine similarities among dehydrogenases of the model organism *E. coli*.

A set of proteins was identified (the same set by both sequence analysis methods), which contains not only a number of NAD-requiring dehydrogenases, but also some NAD-requiring epimerases, dehydratases and isomerases as well. (NAD = nicotinamide adenine dinucleotide.) The latter three reaction types do not involve oxidation/reduction, yet these enzymes require the NAD cofactor. Use of NAD in non-redox reactions is unusual, and it may have been a very early step in evolution to generate a variety of enzyme types from dehydrogenases. A tree of the set of proteins from *E. coli* has two clear groups. One group contains only the NAD-requiring dehydrogenases, while the other contains the NAD-requiring epimerases, dehydratases and isomerases. This is consistent with generation of a set of enzymes from dehydrogenases. This set of enzymes is capable of quite different reactions.

Many other examples of divergence of function from a common sequence are also present in *E. coli*. We did a survey of similarity and function for proteins within *E. coli* and collected 15 examples of pairs (or groups) of 3 or 4 enzymes whose sequences were

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clearly related, but the reactions they catalyze are different. We plan to investigate each case with the view of proposing characteristics of some of the most ancient proteins and their divergent progeny.

HIGHLIGHTS

- Among the Astrobiology Roadmap Objectives are questions about how life began on early Earth, how protocells came into being, and how genomic databases available today can be used to reveal the history and dynamics of evolution. We are analyzing genome data with these objectives in mind.
- We have identified large protein families that are only weakly similar in sequence. However, these families are similar in 3D structure and found in most life forms today. Such families reveal the probable characteristics of very early ancestral proteins.
- Close study also gives information on the mechanisms that led to expansion of biological capability by process of divergence following duplication of genes. Some of the families contain proteins with different functions that evidently diverged from a common starting point. The biochemistry of the commonalities of the diverse functions is a window on the process of divergence. Our goal is to continue to identify members of gene sets and their proteins likely to have been present at early stages of the beginning of life on Earth and sufficient to generate the complexity we have today.

Cross Team Collaborations

Our focus on molecular evolution is very naturally connected with the new Molecular Evolution Focus Group originated by Blair Hedges at the NAI Team at Pennsylvania State University.

Our work is also very naturally connected with the kinds of analysis for closely related proteins done by Steven Benner at the University of Florida and also with the examination of horizontal transfer and protein interactions as elements of evolution, which Jim Lake does at UCLA.

These collaborations have been well discussed among the potential participants and will be undertaken if funding is possible.

Project

Eukaryote Origins and the Evolution of Cellular Complexity – Eukaryotic rRNA Evolution

Senior Project Investigator(s):
M. Sogin, V. Edgcomb, A. Teske

ACCOMPLISHMENTS

EARLY DIVERGING EUKARYOTES (Edgcomb, Sogin, Silberman, Simpson, Patterson)
Pelobionts represent one group of eukaryotes that has been identified as a potentially deep-branching eukaryotic lineage, on the basis of ultrastructure and certain molecular

phylogenies. Pelobionts are flagellated protists that inhabit micro-oxic and anoxic environments. Because they lack stacked dictyosomes, mitochondria, and outer dynein arms in their flagellar apparatus, pelobionts might represent early diverging eukaryotic lineages.

Molecular analyses of DNA-dependent RNA polymerase II suggest the pelobiont *Mastigamoeba invertens* diverged early. Analyses of SSU (small subunit) ribosomal RNAs, in contrast, position pelobionts near the eukaryotic "crown groups," but not as a monophyletic group. We sequenced SSU rRNA genes from four additional pelobiont taxa (including two new isolates). This work confirmed the sequence for *M. invertens* by *in situ* hybridization, compared the ultrastructural features of these taxa, and subjected these new pelobiont sequences to a rigorous phylogenetic analysis in the context of a larger alignment of eukaryotes, including known sequences from other pelobionts.

Results show the pelobionts to be a monophyletic group (with the exception of *M. invertens*), calling into question the taxonomic assignment of this organism and the phylogenetic methodologies that placed it as a deep-diverging eukaryotic lineage. Pelobionts appear to have a sister-group relationship to the entamoebae, to be outside the crown, but they do not represent one of the earliest branching lineages as suggested by RNA polymerase II and ultrastructure.

NOVEL EUKARYOTES AT HYDROTHERMAL VENTS (Atkins, McArthur, Teske)

A cultivation survey of heterotrophic flagellates at Pacific hydrothermal vent sites yielded the flagellate *Ancyromonas sigmoides* at the 9°N East Pacific Rise vents. Molecular and morphological evidence point to *Ancyromonas* as a plausible candidate for the closest relative to the common ancestor of metazoans, fungi, and choanoflagellates (the Opisthokonta), i.e., the origin of multicellularity among animals and fungi (Atkins et al., 2000). Using 18S rDNA (ribosomal DNA) sequences from most of the major eukaryotic lineages (with maximum likelihood, minimum evolution, and maximum parsimony analyses) yielded congruent phylogenies supporting this hypothesis. Combined with ultrastructural similarities between *Ancyromonas* and opisthokonts, the evidence presented here suggests that *Ancyromonas* may form an independent lineage (the Ancyromonadida). This lineage appears to have a closer relationship to the opisthokonts than does the Apusomonadida, its nearest protist relatives.

In addition to these studies, we have nearly completed the analysis of numerous other protist lineages from anoxic environments. Some of these (e.g., retortamonads, *Carpediemonas*, etc.) are basal to other eukaryotes in molecular trees.

HIGHLIGHTS

- Early molecular analyses suggested that the earliest diverging eukaryotes lack mitochondria and peroxisomes. Instead of stacked dictyosomes, these early eukaryotes have a transmembrane golgi-like network.
- Our analyses of ribosomal RNAs show that these characteristics can also be absent in eukaryotic lineages that diverged relatively late. The loss of these organelles has independently occurred several times in the history of eukaryotes.

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#8

Past and Present Life on Mars

Field Expeditions

Several of the organisms in these studies have been isolated from the field, both from marine environments such as Shark Bay (Australia) and from fresh water environments in Queensland (Australia).

Cross Team Collaborations

We have hosted Harvard's paleontological research team headed by Andy Knoll in a broad-ranging discussion about how to link data from the physical record with our most recent studies of protist diversity.

These Harvard-Marine Biological Laboratory joint meetings resulted in a shared agreement to sponsor a year-long visit by David Patterson from the University of Sydney (Australia). Dr. Patterson is a world authority on the evolution of protists and their cytoskeleton. We have also agreed to share the funding of our educational coordinator, Lorraine Olendzenski, after she completes her Ph.D. requirements later this year.

Project

Eukaryote Biodiversity and Physiology at Acidic Extremes: Spain's Tinto River

Senior Project Investigator(s):

M.L. Sogin, L. Amaral Zettler, and R. Amils

ACCOMPLISHMENTS

This project aims to understand how microbial communities thrive under extreme conditions. Our study is based in the Tinto River located in southwestern Spain. The river is a high acid/metal environment with pH values between 2.0-2.5 and iron concentrations as high as 20 g/l. Algae contribute up to 65% of the total biomass of the community. This eukaryotic microbial community is understudied.

Our project explores molecular eukaryotic diversity by looking at SSU rRNA (small sub-unit ribosomal RNA) genes and considers physiological adaptations allowing survival under pH extremes. Sequencing efforts have confirmed a strong algal component in the microbial community. Our molecular data reveal that certain biofilm-communities are dominated by phototrophs such as Chlamydomonas and Chrysophyte populations. Others are a complex assemblage of fungi, ciliate and flagellate populations. Still others are a combination of ciliate and diatom assemblages.

Future work will focus on obtaining sequences from sediment samples from the Tinto River. We will then compare that data with sequences for microbes from other acidic sites such as Davis Mine (an abandoned sulfuric acid mine in Massachusetts) and hot acidic furnas of the Azorean Islands. The physiology component of this project seeks to understand how pH-extremophiles maintain internal pH and if there are different mechanisms by which internal pH is maintained by acidophiles. We have been making internal pH measurements using pH-sensitive indicators such as BCECF (pH-sensitive fluorescent dye) on algal cultures from the Tinto River. Our physiological experiments employ fluorescence ratio imaging and ion-selective probe technology accomplished at

the single-cell level. Initial experiments conducted on cultures of *Chlamydomonas* sp. and *Euglena* sp. (growing at pH 3) revealed striking differences between the two species. Our preliminary results show that *Euglena* sp. may have an internal pH below 6.5. In contrast, the internal pH of *Chlamydomonas* sp. appears nearer neutrality. The putative acidic values of *Euglena* sp. will be confirmed with alternative pH indicators.

HIGHLIGHTS

- In addition to defining the environmental limits of eukaryotic life with respect to pH and heavy metal tolerance, these studies promise to provide insights about the mechanisms that allow eukaryotic acidophiles to adapt to extreme environments.

Field Expeditions

Fieldwork in April 1999: Tinto River, located in the Huelva district of Southwestern Spain.

Dr. Linda Amaral Zettler (Marine Biological Laboratory) collected samples with the assistance and guidance of Dr. Ricardo Amils (Centro de Astrobiología/Universidad Autónoma de Madrid), whose lab has been studying the river for 10 years. Purposes of the expedition were to: (1) obtain sediment and biofilm samples for DNA extraction; and (2) start enrichment cultures of acidophilic algae. These samples were collected for use in physiological experiments to be done in collaboration with Dr. Peter Smith of the BioCurrents Center at the Marine Biological Laboratory in Woods Hole, Massachusetts.

Cross Team Collaborations

We are currently engaged in ongoing collaboration with our colleagues from Spain. As mentioned above, we have already carried out a collection expedition to the Tinto River. As part of this expedition, we did some sample processing out of Dr. Amils' laboratory in Madrid. During this visit, Dr. Amaral Zettler gave a lecture about our project to the molecular biology group at the Autonomous University in Madrid.

As a collaborative exchange for Dr. Zettler's activity in Spain, Dr. Amils and Dr. Gomez visited the Marine Biological Laboratory in the Fall of 1999. Dr. Amils' visit involved giving a presentation on the Tinto River to the Woods Hole scientific community. He also worked on arrangements for a Fulbright Fellowship that would help to facilitate further collaboration between our two groups.

Our collaboration represents the first international cooperation at the NAI and serves to further international and interdisciplinary research in extreme environments. In addition, Dr. Gomez (an Astrobiology Postdoctoral Fellow from Spain) spent a month and a half last fall at the Marine Biological Lab working on bacterial samples from the Tinto River and learning molecular techniques and methods in phylogenetics. Both he and Dr. Amils will be returning to Woods Hole this summer to continue collaboration efforts and prepare manuscripts to summarize the data obtained thus far.

Roadmap Objectives

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Project

Diversity and Physiology of Prokaryotes in Selected Thermophilic and Mesophilic Environments That Might Resemble Early Earth's Biosphere

Senior Project Investigator(s):

A. Teske, V. Edgcomb and H.W. Jannasch

ACCOMPLISHMENTS

Deep-sea hydrothermal vents are potential analogs to subsurface, extraterrestrial microbial habitats. We have been investigating the diversity of microbial populations in organic-rich, hydrothermally heated sediments of Guaymas Basin vent sites (Gulf of California), using a combination of molecular sequencing and characterizations of lipids. The vent fluids have an unusually high carbonate content and support rich bacterial and archaeal diversity. This diversity includes hyperthermophilic methanogens (*Methanococcus*, *Methanopyrus*), sulfate reducers (*Archaeoglobus*), and massive bacterial mats of sulfur-oxidizing, filamentous *Beggiatoa* spp.

We sequenced 100 cloned 16S-rRNA genes from the upper 2 cm of Guaymas Basin sediment core A (*Alvin* Dive 3202, April 1998). The core was covered with a mesophilic, sulfur-oxidizing *Beggiatoa* mat, whereas the sedimentary surface layers contain a homogeneous assemblage of methanogens that are related to archaea from cold marine methane seeps at the Eel River Basin, California. These archaea are candidates for anaerobic methane oxidation, based on their association with diagnostic C-13-depleted archaeal lipids, including high levels of archaeol and 3-hydroxyarchaeol. The Guaymas sediments apparently harbor a novel archaeal community driven by anaerobic methane oxidation. This discovery suggests a sedimentary microbial methane cycle of anaerobic methanotrophs and methanogens that functions without oxygen input. This is a new microbial model system for anoxic extraterrestrial habitats.

The bacterial community is considerably more diverse, but it is dominated by epsilon proteobacterial exosymbionts, as well as delta proteobacterial propionate-oxidizing, sulfate-reducing *Desulfobulbus* and *Desulforhopalus*. The delta-*Proteobacteria* clones are candidates for hydrogenotrophic, sulfate-reducing anaerobes within anaerobic methane-oxidizing consortia. Such anaerobes would scavenge the methane and educt hydrogen, thus making anaerobic methane oxidation thermodynamically possible. This community also includes: (1) green, non-sulfur bacteria; (2) members of the OP11 and OP8 subdivisions originally described in a hot Yellowstone spring; and (3) a collection of gram-positive bacteria, namely the Planctomycetales, the Nitrospira phylum, and the candidate subdivision OP1. Except for the possibly aerobic epsilon-*Proteobacteria*, the clones indicate a predominantly anaerobic, mesophilic bacterial community in the Guaymas sediment surface.

During the next year, we will extend the analysis of core A by at least 1 cm and study the upper 6 cm layers of core C, a sediment core with a less steep temperature profile than core A. Lipid analyses will also follow as practical.

HIGHLIGHTS

- This research has identified yet another novel archaeal community that makes its living through anaerobic methane oxidation.

Field Expeditions

Fieldwork for this study was carried out using the deep-sea submersible, Alvin. The research team included international collaborators who carried out a multidisciplinary study of the core samples. In addition to research on microbial populations, the environmental temperature profiles, density measurements, lipid analyses, pore water, etc. were also studied. All of the data will be correlated in order to provide a detailed description of this unusual hydrothermal environment.

Cross Team Collaborations

Results of these studies were discussed with members of other astrobiology teams at the *First Astrobiology Science Conference* (April 2000) held at Ames Research Center. To date, no formal collaborations have developed, but there are obvious potential ties with the Carnegie Institution of Washington group.

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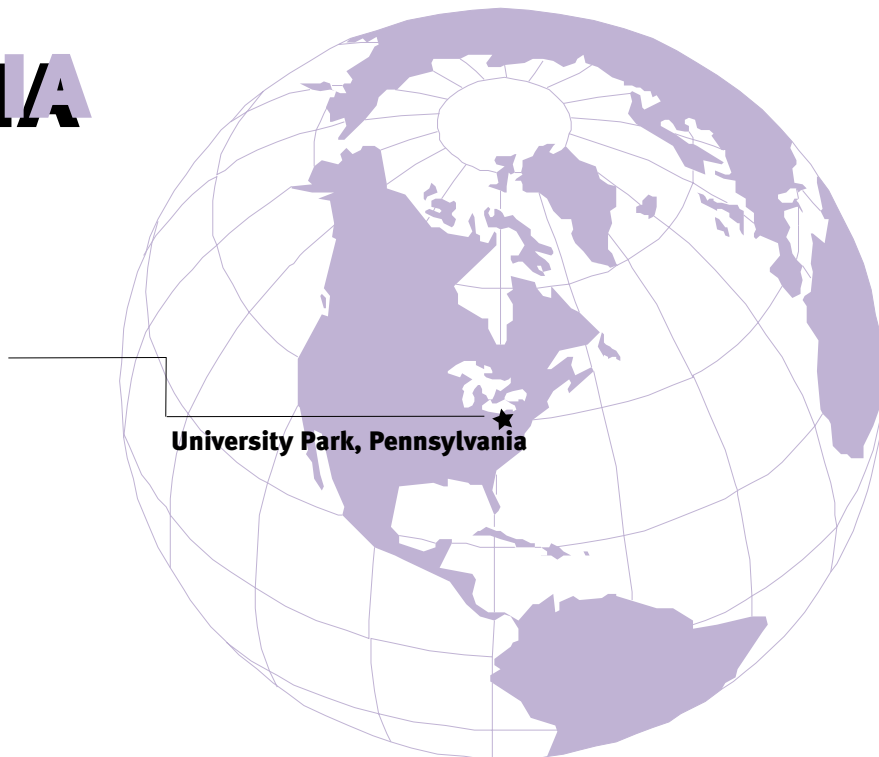
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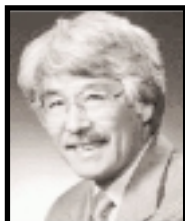
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PENNSYLVANIA STATE UNIVERSITY



PRINCIPAL INVESTIGATOR



**Hiroshi
Ohmoto**

PSU TEAM MEMBERS

Vanessa Amme,
*Pennsylvania State
University*

Michael Arthur,
Pennsylvania State University

Mark Barley,
University Of Western Australia

Hubert Barnes,
Pennsylvania State University

Michael Bau,
Pennsylvania State University

Nic Beukes
Rand Afrikaans University

Jennifer Bland,
Pennsylvania State University

Philip Borkow,
Pennsylvania State University

Birthe Borup,
Pennsylvania State University

Susan Brantley,
Pennsylvania State University

Paul Braterman

Jean Brenchley,
Pennsylvania State University

PROPOSAL EXECUTIVE SUMMARY (1998)

This proposal requests funds from NASA to create The Penn State Astrobiology Research Center (PSARC) as a Member Institution of the NASA Astrobiology Institute (NAI). The primary mission of the PSARC is to conduct multidisciplinary research and public education in astrobiology. The PSARC will be established as an inter-college research organization of The Pennsylvania State University, affiliated also with research groups from The State University of New York at Stony Brook and the University of Pittsburgh.

The major goal of research at the PSARC is to increase our understanding of the connection between the changes in the environment and the change in the biota on Earth, especially during the early stages of its evolution. This will greatly enhance our ability to predict and identify the possibility of life elsewhere in the solar system. Here, we are mainly concerned with the origin of life as well as the evolution and extinction of important organisms, including methanogenic and methanotrophic bacteria, O_2 -producing cyanobacteria, sulfate-reducing and sulfide-oxidizing bacteria, eukaryotes, and early animals. The term "environment" refers specifically to the chemistry of the atmosphere (especially the concentrations of CO_2 , CH_4 , CO , and O_2), the chemical and thermal structure of the ocean (especially its redox state), and climate. The focus of our research will be placed on the geologic period between ~4.5 Ga and 0.4 Ga, which encompasses the time from the emergence of life to the major divergence of animals. The above research goal will be pursued by multidisciplinary research focused on the five research areas outlined below.

I. The Environment of Prebiotic Earth and the Origin of Life: Theoretical and Experimental Approach. Team I. Kasting (Leader), Schoonen, Minard, Arthur, and Ohmoto

Research in Section I will focus on whether the compounds needed for prebiotic synthesis (HCN , NH_3 , H_2CO , H_2CO_2 , and more complex organic compounds such as amino acids) could have been formed within Earth's primitive atmosphere and surface ocean or, alternatively, within submarine hydrothermal systems. We plan to investigate these questions using a combination of theoretical modeling and laboratory experiments.

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<http://psarc.geosc.psu.edu/>

Three specific tasks are proposed for this research area:

1. Task 1 will consist of photochemical modeling to estimate the rates of CH₄ and HCN production in high-CO atmospheres and to determine whether it is possible to form Titan-like hydrocarbon smog. This follows a suggestion by Kasting (1990) that CO, rather than CO₂, may have been the dominant carbon-bearing species in the primitive atmosphere;
2. Task 2 will consist of laboratory experiments to determine the rate at which CO hydrates to form formate (HCO₂), along with the rate at which formate disproportionates to form other organic compounds. These factors must be understood in order to predict whether CO was actually abundant in the early atmosphere;
3. Task 3 will more generally evaluate the rates of synthesis of prebiotic C-N-O-H compounds in hydrothermal vent environments. Specifically, we will determine: i) if the reduction of N₂ to NH₃ can proceed at hydrothermal temperatures via metal sulfide-catalyzed reaction pathways; and ii) if C-O-H and C-N-O-H species can be formed in systems buffered by the mineral assemblage pyrite-pyrrhotite-magnetite.

II. Roles of Metals in the Origin and Evolution of Life: Microbiological and Biochemical Approach. Team II. Ferry (Leader), Brenchley, and Brantley

Methanogenic bacteria are thought to be among the first organisms to have evolved on Earth, based on their position near the base of evolutionary trees derived from molecular phylogeny. Ferry (1995, 1997) has discovered that these bacteria contain an enzyme (CO dehydrogenase/acetyl-CoA synthase or CODH/ACS), which non-photosynthetically fixes CO and CO₂ into acetic acid. Additionally, Ferry has found that this enzyme contains a Ni-X-[4Fe-4S] metal center (where X is an unidentified bridging atom).

These discoveries mesh well with Kasting's model for a CO-dominated early atmosphere and with Wachtershauser's theory for the role of Ni-Fe-S minerals in prebiotic synthesis. Thus, the CODH/ACS enzyme may contain important clues about the nature of the first life on Earth, as well as the nature of the primitive environment. The research in Section II is directed toward: 1) investigation of the structure, function, and assembly of CODH/ACS and other metalloenzymes; 2) investigation of interactions between

Jochen Brocks,

AUSTRALIAN GEOLOGICAL SURVEY ORGANIZATION

Lisa Brown,

Pennsylvania State University

Tom Bullen

Rosemary Capo,

University Of Pittsburgh

Oliver Chadwick,

University Of California Santa Barbara

Hsiong Chen,

Pennsylvania State University

Christopher Coath,

University Of California Los Angeles

David Crown,

University Of Pittsburgh

Linda Decker,

Pennsylvania State University

Catherine Drennan,

Massachusetts Institute Of Technology

Amy Eastwood,

University of Virginia

Brooke Eidell,

Pennsylvania State University

Jennifer Eigenbrode,

Pennsylvania State University

James Ferry,

Pennsylvania State University

Marc Fiddler,

Pennsylvania State University

Charles Fisher,

Pennsylvania State University

Sorel Fitz-Gibbon,

University Of California Los Angeles

Tracey Frank,

University Of Queensland

Katherine Freeman,

Pennsylvania State University

Brian Games,

University of Pittsburgh

David Geiser,

Pennsylvania State University

Galina Glazko,

Pennsylvania State University

Robert Graham,

University Of California Riverside

Kathy Gross,

Pennsylvania State University

Robin Guynn,

Pennsylvania State University

William Harbert,

University Of Pittsburgh

T. Mark Harrison,

University Of California Los Angeles

Ken-ichiro Hayashi,

Tohoku University

Peter Heaney,

Pennsylvania State University

Daniel Heckman,

Pennsylvania State University

Blair Hedges,

Pennsylvania State University

methanogenic microbes and Ni-containing sulfides, as well as other Ni-containing minerals; and 3) culturing and isolation of psychrophilic organisms capable of fixation of CO and CO₂ and containing CODH/ACS.

III. Timescale for the Early Evolution of Life on Earth: Molecular Evolutionary Approach Team III. Nei (Leader) and Hedges

Research in Section III will focus on estimating the times of origin and early events of evolution for life on Earth by using information on the divergence of protein and DNA sequences between extant organisms. In order to accomplish this goal, efficient statistical methods for constructing molecular clocks and for evaluating the reliability of the molecular time estimates will be developed for use when large numbers of genes from diverse organisms are used.

Also to accomplish our research goal, a large amount of sequence data useful for estimating divergence times will be compiled. These data will be taken from the literature or generated in the laboratory for diverse organisms representing major kingdoms and phyla as well as the three kingdoms (domains): eubacteria, archaeobacteria, and eukaryotes. Sequence data will also be obtained for representative vertebrate species, for which reliable fossil records are available, in order to calibrate molecular clocks. For this purpose, approximately 3,500 new genes will be sequenced. The protein or DNA sequences obtained will then be subjected to statistical analyses to estimate the times of origin of proto-organisms and the divergence of major groups of extant organisms. The final step will be to find links between the times of major events of evolution of life and those of geochemical and atmospheric evolution. A database of molecular time estimates for storage, retrieval, and further analysis will also be established for public use.

IV. Evolution of Atmospheric O₂, Climate, and the Terrestrial Biosphere: Approach From Field-Oriented Geochemical Investigations. Team IV. Kump (Leader), Ohmoto, Freeman, Arthur, Capo, and Stewart

Two contrasting models exist for the time history of atmospheric O₂ and its relationship with biological evolution. The Cloud-Walker-Kasting-Holland model postulates that PO₂ rose from ~10-13%PAL (present atmospheric level) to >15% PAL during the period between 2.2 and 1.9 Ga. This was followed by a second major rise in PO₂ to nearly the present level between ~1.0 Ga and 600 Ma. An implication of this theory is that O₂ increases were responsible for the appearance of various organisms, such as: (1) eukaryotes and sulfate-reducing bacteria, appearing at ~2.0 Ga; and (2) metazoa and sulfide-oxidizing bacteria, appearing at ~600-800 Ma.

By contrast, the Dimroth-Ohmoto model postulates an essentially constant atmospheric O₂ level (within ±50% of PAL) since ~4.0 Ga. In this case, the appearance of new organisms would be independent of atmospheric PO₂. We propose to test these two models by performing detailed geochemical investigations of a variety of Precambrian rock types, including paleosols, uraniferous conglomerate beds, redbeds, marine shales, banded iron-formations, cherts, and carbonates. These studies will focus on micro-scale

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and molecular-level variations in mineral textures, as well as chemical and isotopic compositions. Along with O₂, we will also attempt to constrain the time history of atmospheric CO₂ and its effect on continental weathering.

V. Causes and Consequences of the Diversification and Extinction of Metazoans: Field, Geochemical and Paleontological Investigations. Team V. Arthur (Leader), Patzkowsky, Freeman, Kump, Ohmoto, Capo, and Stewart

The advent and continuity of complex organisms on Earth is of great interest because of the tenuous hold that such life forms must have had in the rapidly evolving global environment. A key goal is to develop an understanding of the impact of tectonically- and extraterrestrially-driven environmental change on early organisms, as well as the effect that early organisms had on their environment.

Our proposed research is divided into two different tasks:

1. Task 1 will focus on the evolution of marine metazoans during the Riphean through early Cambrian (850-520 Ma). In particular, we will attempt to test the hypothesis of Canfield and Teske (1996). They hypothesize that sulfide-oxidizing bacteria became important in the Riphean as a consequence of an increase in atmospheric O₂. We will accomplish this task by performing detailed chemical and isotopic analyses of continuous sedimentary sequences deposited during this time interval.

2. Task 2 will be to investigate the causes and consequences of six Cambrian and Ordovician extinction events, including the Late Ordovician mass extinction. We propose to generate an integrated data set focused on this time period, both to enhance understanding of environmental causes of diversification and extinctions and to develop a self-consistent theory to explain the observed extinction events. Our study will differ from previous investigations in that our linked set of geochemical, stratigraphic, and paleontological data will permit us to determine whether patterns of extinctions are consistent with hypothesized extinction scenarios.

University Commitment and Public Outreach

To aid us in achieving the above research goals, Penn State University and the College of Earth and Mineral Sciences have pledged to contribute a total of \$260K-\$280K per year. These monies will be used to fund two new faculty positions in astrobiology-related fields, 1 postdoctoral fellowship, 2 graduate fellowships, and 1 graduate research assistant, as well as to support undergraduate education and provide matching funds for equipment.

Educational options in astrobiology will also be created for both the undergraduate and graduate programs. An additional \$100K per year (\$60K per year from Penn State and \$40K per year from NASA) will be used to support our training/public outreach program. This program will include cooperative efforts with the Pennsylvania Space Grant Consortium and with the Penn State WISE (Women in Science and Engineering) Program to attract promising high school and junior high school students and to educate middle school science teachers. We will also develop a Penn State astrobiology web site, as well as an astrobiology exhibit at the Carnegie Museum of Natural History in Pittsburgh.

Steven Holland,
University Of Georgia

Roberta Hotinski,
Pennsylvania State University

Christopher House,
Pennsylvania State University

Matthew Hurtgen,
Pennsylvania State University

Sanae Ikeda,
Pennsylvania State University

Kim Kahle,
Pennsylvania State University

Takeshi Kakegawa,
Tohoku University

Birgitta Kalinowski

James Kasting,
Pennsylvania State University

Alan Kaufman,
University Of Maryland

Brian Krapez,
University Of Western Australia

Sudir Kumar,
Arizona State University

Lee Kump,
Pennsylvania State University

Andrew Kurtz,
Pennsylvania State University

Laura Liermann,
Pennsylvania State University

Kristy Longsdorf,
Pennsylvania State University

Donald Lowe,
Stanford University

James Lyons,
University Of California Los Angeles

Timothy Lyons,

University Of Missouri Columbia

Maureen Lyons-Weiler,

Pennsylvania State University

Gwendolyn Macpherson

Kevin Mandernack

J Martini,

Geological Survey Of South Africa

Clifford Matthews,

University Of Illinois

Kevin McKeegan,

University Of California Los Angeles

Arnold Miller,

University Of Cincinnati

Robert Minard,

Pennsylvania State University

Joseph Minervini,

University Of Pittsburgh

Fayek Mostafa

Hiroshi Naraoka,

Tokyo Metropolitan University

Munetomo Nedachi,

Kagoshima University

Masatoshi Nei,

Pennsylvania State University

Kathleen O Toole,

Pennsylvania State University

Hiroshi Ohmoto,

Pennsylvania State University

Bertil Olsson,

Pennsylvania State University

Shuhei Ono,

Pennsylvania State University

We believe that the research proposed by the Penn State Astrobiology Research Center will contribute significantly to the overall goals of the NASA Astrobiology Institute by expanding our understanding of the origin and early evolution of life and its interaction with planetary environments. We feel that we are uniquely qualified to address these aspects of astrobiology because of the combined expertise of our research group at Penn State and our Co-PI's and collaborators at other institutions. We also believe that we can have a significant impact on public awareness of astrobiology and that we can attract substantial numbers of new students to the field. We look forward to participating in this exciting new intellectual experience.

YEAR 2 EXECUTIVE SUMMARY

PSARC is composed of 16 Full Members (PI and Co-I's) who are full-time faculty members of Pennsylvania State University, the University of Pittsburgh, or the State University of New York at Stony Brook, plus their research staff (post-doctoral fellows, graduate and undergraduate students, technicians), and administrative staff. During the second year with NAI, a large number of people have been involved in the research activities of the PSARC, including 8 Research Associates, 2 Research Assistants, 21 graduate students, 16 undergraduate students, 2 technicians, 1 Administrative Assistant, and 1 web-site designer. We also have 26 active Associate Members who are collaborating with the Full Members, as well as 12 other researchers working with our Co-Investigators. Currently, we have 4 Associate Members who are in Education and Public Outreach, also supporting PSARC activities.

The main goal of research at PSARC has been to increase our understanding of the connection (interplay) between the environment and the biota on Earth, especially during the early stage of its evolution. Attainment of this goal will greatly enhance our ability to predict and identify life elsewhere in the solar system. Here, we are mainly concerned with the origin of life and the evolution and extinction of important organisms (e.g., methanogenic bacteria, cyanobacteria, eukaryotes, terrestrial organisms, and early animals). The term "environment" refers specifically to the chemistry of the atmosphere (especially, the concentration of CO₂, CH₄, CO, and O₂), the chemical and thermal structures of oceans, and climate.

The above goal has been pursued primarily from multidisciplinary and multidimensional research focused on the five task areas listed below.

Task I. The environment of prebiotic Earth and the origin of life: theoretical and experimental approach. J. Kasting (Leader), M. Schoonen, R. Minard, and H. Ohmoto.

Task II. Biochemistry of Archea, focused on the roles of metals in the origin and evolution of life on Earth: Microbiological and biochemical approach. J. Ferry (leader), J. Brenchley, S. Brantley and C. House.

Task III. Timescale for the early evolution of life on Earth: molecular evolutionary approach. B. Hedges (leader) and M. Nei.

Task IV. Evolution of atmospheric O₂, climate, and the terrestrial biosphere: approaches from field-oriented geochemical investigations. L. Kump (leader), H. Ohmoto, K.

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Freeman, M. Arthur, R. Capo, and B. Stewart.

Task V. Causes and consequences of the diversification and extinction of metazoans: field, geochemical and paleontological investigations. M. Arthur (leader), M. Patzkowsky, K. Freeman, L. Kump, H. Ohmoto, R. Capo, and B. Stewart.

Progress in each of the above areas is reported separately in the following pages. Excellent progress has been made in all phases of our research projects.

A total of 25 papers related to astrobiology, excluding those in press, were published by the PSARC members during the second year with the NAI. The PSARC members also gave 29 presentations at the *First Science Conference on Astrobiology* (April 2-5, 2000) held at NASA Ames Research Center (California) and more than a hundred presentations at various other international/national meetings.

HIGHLIGHTS

- For highlights of activities in research, education and public outreach by the individual members, note these points in the reports from our individual members.
- Hiring Dr. Christopher House as Assistant Professor of Astrobiology in the Department of Geosciences at Pennsylvania State (January 2000) is an important development. He has joined PSARC as our 16th PI, indicating the commitment made by Pennsylvania State University to support PSARC.
- Dr. House (who received undergraduate education at UC-San Diego under Stanley Miller and graduate education at UCLA under Bill Schopf and Bruce Runnegar) is the first Ph.D. in astrobiology. He heads our newly created undergraduate minor program of astrobiology and is expected to play a key role in linking geosciences and microbiology and in promoting collaboration among NAI Teams.

Field Expeditions

Several field expeditions were carried out during the period of May, 1999 – August, 1999 to observe the field occurrences of a variety of rocks formed 3.5-2.0 Ga (billion years) ago. We systematically collected these samples for a variety of geochemical investigations aimed at understanding the connection between the evolution of environment (e.g., O₂ and CO₂ contents of the atmosphere and ocean) and the evolution of biota in the oceans and lands in the early history of Earth.

Field expeditions this year were: (1) the Elliot Lake District, Ontario, Canada; (2) the Thunder Bay and Steep Rock Districts, Ontario; (3) the Val D'Or District, Quebec and the Abitibi District, Ontario, Canada; and (4) the Hamersley and Pilbara districts, Western Australia.

A field expedition was also carried out in the Adelaide Geosyncline in South Australia to study Neoproterozoic carbonates.

Field expeditions next year (our third year with NAI) will include: (1) Proterozoic rocks in Finland; (2) Late Archean – early Proterozoic sequence in the Hamersley-Pilbara district in Australia; (3) Paleosols and banded iron formations in the Abitibi belt, Canada; and (4) Neoproterozoic sequence in Arizona – California.

Mark Patzkowsky,
Pennsylvania State University

Alexander Pavlov,
Pennsylvania State University

Robin Penfield,
SUNY Stony Brook

Amanda Pfaff,
Pennsylvania State University

Angela Phelps,
Pennsylvania State University

Helen Pointkivska,
Pennsylvania State University

Laura Poling,
Pennsylvania State University

Anthony Prave,
University Of St. Andrews

Victoria Pretti,
University Of Pittsburgh

Douglas Rees,
California Institute Of Technology

Greg Retallack,
University Of Oregon

Amanda Reynolds,
University Of Pittsburgh

John Rimmer,
Pennsylvania State University

Igor Rogozin,
Pennsylvania State University

Jessica Schendel,
University Of Puget Sound

Trent Schindler,
Pennsylvania State University

Martin Schoonen,
SUNY Stony Brook

J. William Schopf,
University Of California Los Angeles

Peter Sheridan,

Pennsylvania State University

Jason Shoe,

Pennsylvania State University

Kerry Smith,

Pennsylvania State University

Sherry Stafford,

University Of Pittsburgh

Emily Stauffer,

Indiana University

Karl Stetter,

Universität Regensburg

Brian Stewart,

University Of Pittsburgh

Kyle Straub,

Pennsylvania State University

Neil Suits,

Pennsylvania State University

Roger Summons,

Australian Geological Survey Organization

Amanda Thompson,

Pennsylvania State University

Marcel van Tuinen,

Pennsylvania State University

Judi Wakhungu,

Pennsylvania State University

Dennis Walizer,

University Of Pennsylvania

Hidemi Watanabe,

University of Tokyo

Yumiko Watanabe,

Pennsylvania State University

Richard Wilkin,

Pennsylvania State University

Shaole Wu,

Pennsylvania State University

Project

The Environment of Prebiotic Earth: Theoretical Approach

Senior Project Investigator(s):

James F. Kasting

ACCOMPLISHMENTS

1. My graduate student (Alex Pavlov) has been working with the CH₄ photochemical model on a related project. (This model was originally developed by Lisa Brown.) We are trying to determine whether a hydrocarbon aerosol screen could have allowed photosynthetically-produced O₂ to accumulate to significant levels during the Late Archean. Alex has performed all the necessary Mie scattering calculations and is in the process of incorporating them into the climate and photochemical models.

2. My grad student Trent Schindler has completed a paper on synthetic spectra of Earth-like planetary atmospheres. This is only tangentially related to the goals of our proposal, but it relates to the photochemical modeling that Alex has been doing.

TASK I: Comparison with Stated Goals

Originally, Martin Schoonen and I proposed to study the fate of CO produced by impacts and volcanism on the prebiotic Earth. Our thought was that CO would be the dominant carbon-bearing gas produced by large impacts and that CO could also be the dominant carbon-bearing gas emitted by surface volcanoes, if the upper mantle was originally more reduced.

Recently, at the April 3-5, 2000 Astrobiology Science Conference, Monika Kress and Chris McKay presented a poster paper in which they argued that CH₄ and CO₂, rather than CO, would have been the dominant carbon-bearing gases produced by impacts. Their idea is that vaporized metals from the impactor would have catalyzed the conversion of CO into CH₄ as the impact plume cooled. If they are right, then it may behoove us to spend more time concentrating on CH₄ photochemistry and less on CO. This is not necessarily bad from the standpoint of prebiotic synthesis. CH₄ is a critical starting ingredient for the hypotheses in which life originates in surface environments (as opposed to synthesis at deep-sea vents, which was the idea espoused in our original proposal).

Future Plans

Alex and I are also working with Janet Siefert to develop some simple models of a hypothetical Archean ecosystem. Our goal is to try to understand whether one could have an ecosystem on a largely anaerobic Earth: (1) that would produce sediments consistent with the C- and S-isotopic records; and (2) that might possibly explain a transition to higher atmospheric O₂ levels in the early Proterozoic. I will probably hand this project over to a new graduate student, Pusher Khareschka, in the Fall of 2000.

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Meanwhile, Trent is currently working on modeling hydrodynamic escape of hydrogen from the early Earth. Although this work is funded separately (through exobiology), it ties in closely with our modeling of methane-rich atmospheres and with the whole question of the rise of atmospheric O₂.

HIGHLIGHTS

- Expecting some interesting results soon in our work to develop some simple models of a hypothetical Archean ecosystem (although Alex and I have no headlines at this time).

The Environment of Prebiotic Earth and the Origin of Life: Experimental Approach

Project

Senior Project Investigator(s):
Martin Schoonen

ACCOMPLISHMENTS

Our group at SUNY-Stony Brook has focused on two research projects: (1) rate of CO hydration and stability of formate; and (2) reduction of dinitrogen to ammonia, catalyzed by minerals.

The experimental study of the rate of CO hydration is nearly complete, and a new rate equation has been derived for this process. This rate equation will be used by Jim Kasting in his effort to model the fate of atmospheric CO on early Earth. In the second year we have also started to work on the stability of formate. Exposure to light in the absence and presence of minerals has been studied. We can demonstrate that in the presence of several minerals, formate transforms to formaldehyde. This is relevant because it indicates a reduction of formate.

We have also been successful in demonstrating that dinitrogen can be reduced in the presence of iron monosulfide and hydrogen sulfide to ammonia at temperatures ranging from 120°C to 150°C in an all-liquid system. These experiments have been very difficult to conduct because of parallel reactions involving the metal container surfaces. Now that we have been able to overcome this hurdle, we have been able to demonstrate that small amounts of ammonia can be formed under conditions that are relevant for hydrothermal submarine vents. To our knowledge, this is the first study to show this. Both of these projects were included in the original proposal.

Plans for next year involve:

- (1) a continuation of experimental work to determine the stability of formate as a function of exposure to UV, in the presence and absence of minerals
- (2) finishing the work on dinitrogen reduction promoted by FeS/H₂S. We will then derive rate data to quantify this process and explore the role of other minerals.

Ping Xu,
Pennsylvania State University
Yong Xu,
SUNY Stony Brook

Kosei Yamaguchi,
Pennsylvania State University

Roadmap Objectives

- #1 Sources of Organics on Earth
- #2 Origin of Life's Cellular Components
- #3 Models for Life
- #5 Linking Planetary & Biological Evolution
- #7 Extremes of Life
- #12 Effects of Climate & Geology on Habitability

Roadmap Objectives

#1

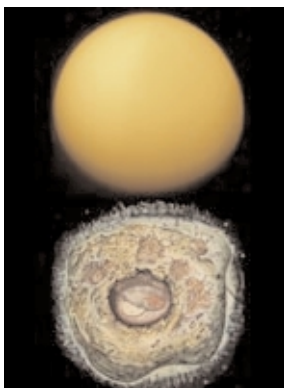
Sources of Organics on Earth

#2

Origin of Life's Cellular Components

#9

Life's Precursors & Habitats in the Outer Solar System



HIGHLIGHTS

- First evidence for a mechanism to reduce dinitrogen to ammonia at conditions relevant to submarine vent systems.
- Formation of ammonia is possibly the first step in making amino acids and other more complex compounds.

Project

The Prebiotic Chemistry of Hydrogen Cyanide

Senior Project Investigator(s):
Robert D. Minard

ACCOMPLISHMENTS

Research by undergraduates Emily Stauffer and Kim Kahle, particularly in the last two summers, has demonstrated that a Titan atmospheric chemistry simulation product ("tholin") yields a suite of thermal/hydrolytic degradation products that are very similar in composition to the mixture of degradation products from HCN polymer. This work was done using a new method for analyzing high molecular weight macromolecular materials called tetramethylammonium hydroxide (TMAH) thermochemolysis GC-MS (gas chromatography-mass spectrometry) (Minard et al., 1998). This provided strong support for the concept that HCN chemistry is an important part of the atmospheric chemistry of Titan and that HCN polymer is a major component of the orange-colored haze in that atmosphere. This work was reported in an Origin of Life Symposium in the Geochemistry Division at the American Chemical Society Meeting in San Francisco on April 4th, 2000 and should appear in an issue of Geochemistry (Minard et al., 2000).

Research by undergraduate Vanessa Amme in the summer of 1999 attempted to follow the initial steps of HCN polymerization by HPLC, NMR, and APCI-MS. Most significant was the development of a method to produce HCN trimer (aminomalononitrile), in order to study its oligomerization reactions using these methods.

Collaborative work with Paul Braterman involves the examination of HCN polymerization products formed on aluminum magnesium-layered double hydroxide mineral analogues.

Analytical methodology was developed to look at the products from carbon monoxide hydration in collaboration with Martin Schoonen.

Undergraduate Marc Fiddler has worked on improving methods for carrying out tetramethylammonium hydroxide thermochemolysis on macromolecular materials such as tholins, HCN polymer, and meteoritic organic matter. He will continue the work this summer.

In the upcoming summer, four undergraduates (Eastwood, Fiddler, Longsdorf, and

Year 2

Schendel) will continue studies aimed at unraveling the macromolecular structures of materials relevant to the origin of life. Such study materials include HCN polymer, tholin, and other prebiotic chemistry simulation products, plus organic material in meteorites. Studies of these materials use various analytical techniques described here for our research. In addition, a graduate student in Dr. Karl Mueller's research group, Kathy Gross, will start $^{13}\text{C}/^{15}\text{N}$ solid state NMR (nuclear magnetic resonance) studies of these materials.

In August, a student (Robin Penfield) from Martin Schoonen's lab at SUNY-Stony Brook will come to our labs at Penn State to use the GC-MS system to search for acetic and higher organic acids formed in her CO hydration studies.

HIGHLIGHTS

- Results to date have led to the hypothesis that HCN polymers (consisting of a polyamide or polyamidine backbone with a variety of pendant purine, pyrimidine, or other N-heterocyclic side groups) could have the structure, size, and heterogeneity necessary for both molecular recognition (catalysis and base pairing) and information storage.
- If the above situation is the case, then HCN polymers may represent the first true biopolymer capable of self-replication, with resulting evolution and differentiation into a concomitant "Protein-RNAWorld."
- The postulated spontaneous direct formation of a primordial biopolymer with biologically-relevant subunits would, in a single stroke, eliminate the myriad difficulties of concentrating and condensing monomeric "building blocks" in the primitive ocean.

Enzymes of Ancient Metabolic Pathways

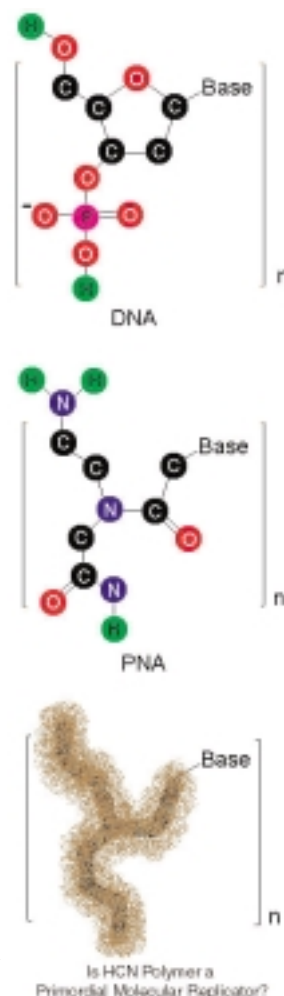
Project

Senior Project Investigator(s):
James G. Ferry

ACCOMPLISHMENTS

Part 1

It is unknown how the Archaea fix sulfur or synthesize cysteine. We report the purification and characterization of OASS (O-acetyl-serine sulfhydrylase) from acetate-grown *Methanosarcina thermophila*, a moderately thermophilic methanoarchaeon. The purified OASS contained pyridoxal 5'-phosphate and catalyzed the formation of L-cysteine and acetate from O-acetyl-L-serine and sulfide. The N-terminal amino acid sequence has high sequence similarity with other known OASS enzymes from the Eukarya and Bacteria domains. The results of this study provide the first evidence, of any kind, for a sulfur-fixing enzyme in the Archaea domain. The results also provide the first biochemical evidence for an enzyme with the potential for involvement in cysteine biosynthesis in the Archaea.

Roadmap
Objectives

#2

Origin of Life's Cellular
Components

Carbonic anhydrases catalyze the reversible hydration of CO_2 and are ubiquitous in highly evolved eukaryotes. We report that carbonic anhydrase is widespread in the Archaea and Bacteria domains and is an ancient enzyme. This occurrence in chemolithoautotrophic species, occupying deep branches of the universal phylogenetic tree, suggests a role for this enzyme in the proposed autotrophic origin of life. We also report on the first known plant-type (β class) carbonic anhydrase in the Archaea. The *Methanobacterium thermoautotrophicum* ΔH *cab* gene was hyperexpressed in *Escherichia coli*, and the heterologously produced carbonic anhydrase was purified 13-fold to apparent homogeneity. These results show that β carbonic anhydrases extend not only into the Archaea domain, but also into the thermophilic prokaryotes.

Future work will further explore the pathways for sulfur fixation in the Archaea. The presence of new enzymes involved in sulfur assimilation and cysteine biosynthesis in *Methanosarcina thermophila* will be sought. Genetic approaches including gene knock out experiments will be carried out to validate the involvement of enzymes in proposed pathways.

Part 2

The origin of life occurred sometime between 3.8 (end of heavy bombardment of Earth) and 3.5 (presence of microfossils in ancient sediments) billion years ago. In the absence of a volcanic source of methane and ammonia, the Earth's atmosphere was a weakly reduced mixture of CO_2 and N_2 combined with lesser amounts of CO , H_2 , and reduced S gases (Kasting, 1993). The yield of complex organic matter through abiotic reactions under these conditions is believed to have been extremely low (Kasting, 1997). However, experiments by Wachtershauser (1997) found that an aqueous slurry of co-precipitated NiS and FeS converted CO and CH_3SH into acetic acid.

This research opened up the possibility of a chemoautotrophic origin of life in which early organisms could fix CO_2 and CO enzymatically. Carbon monoxide dehydrogenase/acetyl CoA synthase (CODH/ACS), the central enzyme of the reductive acetyl CoA pathway of many diverse modern-day anaerobes, fixes CO and CO_2 non-photosynthetically. CODH/ACS utilizes substrates (CO , H_2 , and CO_2) proposed to be present in the Earth's atmosphere around the time life originated. Its active site contains a NiFeS metal center, similar to that demonstrated in the experiments by Wachtershauser

Our goal this past year was to get a better understanding of the structure and the formation of this important ancient enzyme. Crystal trials are currently in progress for the structure determination of the CODH/ACS from *Methanosarcina thermophila*, in collaboration with Dr. Doug Rees of Cal Tech.

Formation of this important ancient enzyme and its NiFeS active site requires cysteine. Through genetic, physiological, and biochemical studies, we determined that cysteine biosynthesis occurs through the serine pathway in *Methanosarcina thermophila*. The two genes required for this pathway form an operon in *M. thermophila*. OASS (O-acetylserine sulfhydrylase), which catalyzes the final step of the pathway, exhibits positive cooperativity and is expressed under growth conditions requiring cysteine biosynthesis. The last two genes of the homoserine pathway do not seem to be present in



Structure of Cab, the beta class carbonic anhydrase from the thermophilic archaeon *Methanobacterium thermoautotrophicum*.

The dimer structure of Cab is presented with monomer A in magenta and monomer B in blue. HEPES bound to subunit A is shown in ball-and-stick presentation. The active site zinc for each monomer is displayed in grey.

Year 2

the genome, and the enzymes are not expressed under growth conditions requiring cysteine biosynthesis.

Future work will entail getting crystals of CODH/ACS for X-ray crystallography. The two genes of the serine pathway will be knocked out individually, in order to determine if the serine pathway is the only pathway present for cysteine biosynthesis in *M. thermophila*.

Biochemistry of Psychrophilic Organisms

Project

Senior Project Investigator(s):
Jean Brenchley

ACCOMPLISHMENTS

One objective of the work was to test the proposal that all organisms placed phylogenetically at the base of the tree of life are thermophiles. Such work is important to study the current view of the existence of thermophiles as deep-branching organisms. This outlook has been used as evidence that the Last Common Ancestor was a thermophile and that the early Earth environment was therefore hot. Another possibility is that deep-branching psychrophiles exist, but they have not been isolated.

We have sampled a Greenland Ice Core and have turbid cultures in chemoautotrophic anaerobic media incubated at 0°C. We are attempting to increase the cell yield in order to extract sufficient DNA for PCR (polymerase chain reaction) amplification to determine the types of microorganisms that are present. Independent of whether the organisms are deep branching, the existence of these organisms is important for interpreting the geochemical analysis of the ice cores.

Another aspect of our work has been to isolate and characterize novel psychrophilic organisms as a basis for understanding how life might exist in other extreme environments. As part of this work, Dr. Sheridan has isolated several psychrophilic bacteria including a *Planococcus* species from a melt pond in Antarctica. He cloned and characterized a gene encoding a beta-galactosidase from this isolate. This gene is a member of the family 42-glycosyl hydrolases and produces a salt-tolerant beta-galactosidase that might be useful as a reporter gene in halophilic organisms. A manuscript describing this work will be published in the June 2000 issue of *Applied and Environmental Microbiology*.

During the next year we will: (1) continue the attempts to grow, isolate, and characterize the anaerobes from the ice cores; and (2) continue our characterization of new psychrophilic organisms and their cold-active enzymes as models for life in cold environments elsewhere.

Roadmap Objectives

#2

Origin of Life's Cellular Components

#4

Genomic Clues to Evolution

#5

Linking Planetary & Biological Evolution

#6

Microbial Ecology

#7

Extremes of Life

Roadmap Objectives

#6

Microbial Ecology

#7

Extremes of Life

#8

Past & Present Life on Mars

Project

Microbe-Mineral Interactions

Senior Project Investigator(s):

S.L. Brantley

ACCOMPLISHMENTS

Although the average crustal abundances and surface water concentrations of Fe, Mn, Zn, Ni, Cu, Co, and Mo are extremely low, each of these metals is used in bacterial enzymes, coenzymes, and cofactors. While it is well known that bacteria excrete siderophores to extract Fe from their environment, it is not understood how these siderophores attack minerals to provide the Fe^{III}. It is also not understood how bacteria extract other micronutrients.

We are investigating how soil microbes extract these metals from common minerals. Our work has progressed most rapidly with investigations of hornblende. Leaching of metals from hornblende by microbes is being investigated in order to determine: 1) the mechanisms of metal mobilization by soil microbes; 2) whether natural mineral surface chemistry documents biotic metal extraction; and 3) whether iron isotope ratios of leached Fe is diagnostic of bacterial activity.



Arthrobacter growing on the surface of hornblende.
Scale bar is shown.

Isolates from an Adirondack soil containing hornblende were cultured in enriched medium. Partial sequencing of the 16S rRNA (16S ribosomal RNA) gene of two aerobes capable of mobilizing Fe from hornblende showed that the two isolates are probably a streptomycete and an arthrobacter. (16S rRNA gene source: Nucleic Acid Facility, Life Sciences Consortium, Pennsylvania State University). In buffered media, the streptomycete and arthrobacter significantly increased the Fe release rate from hornblende over abiotic controls. Two different catechol siderophores produced by the isolates and characterized by HPLC (high performance liquid chromatography) and MS (mass spectrometry) are presumed to cause this Fe release enhancement. X-ray photoelectron spectroscopy (XPS) of hornblende planchets after dissolution in the presence of the arthrobacter revealed a substantial drop in the Fe/Si ratio of the hornblende surface after removal of the bacteria, as compared to control samples treated in media without bacteria.

HIGHLIGHTS

- Isotopic composition (⁵⁶Fe/⁵⁴Fe) of Fe in solution after incubation of Fe-containing silicate with a soil microbe is approximately 1 per mil lighter than Fe released to solution from the same mineral abiotically without organic ligands present.
- Fe released to solution, from hornblende dissolved abiotically with organic ligands present, is also fractionated.
- Fe isotopes may therefore be useful, along with surface spectroscopies such as XPS (X-ray photoelectron spectroscopy), to identify evidence on weathered minerals indicative of metal extraction due to bacteria or organic ligands.

Year 2

Whole Genome-Based Phylogenetic Analysis of Free-Living Microorganisms

Project

Senior Project Investigator(s):
Christopher House

ACCOMPLISHMENTS

This project is aimed at using whole genome sequences to understand the Tree of Life. The project has made great progress during the reporting period. The project was initiated in late 1998, and the project soon had its first robust Tree of Life using super families of gene sequences as phylogenetic characters.

During early 1999, the tree was analyzed in depth and expanded to include new genome sequences available. The results were published in the November 1999 issue of *Nucleic Acids Research*. Since publication of this first paper, the project was been expanded to include additional published genome sequences. Our work on this was featured prominently in a perspectives article published in *Science* in December 1999 (Banfield and Marshall, 1999). We consider the progress to date to be excellent and on schedule.

Currently, we are preparing a second manuscript that explores microbial relationships in more detail and compares our results with those from other techniques. After this publication, we hope to use our life tree (based on genome sequences) as a baseline on which to map the transfer of genes between different microbial groups.

HIGHLIGHTS

- The creation of a robust Tree of Life based on genome sequences, which is very similar to the rRNA tree.
- Publication in November 1999 *Nucleic Acids Research*
- Work featured in December 1999 *Science* publication

Paleomicrobiology and the Evolution of Metabolic Pathways in the Archean Environment

Project

Senior Project Investigator(s):
House, Schopf, Harrison, and Stetter

ACCOMPLISHMENTS

The project has two parts. First, ancient microfossils preserved in cherts are studied with an ion microprobe to analyze the carbon isotopic composition of individual microfossils. Second, the carbon isotopic fractionation process is studied in a diverse set of microorganisms. Collectively, the results will constrain the carbon fixation biochemistry of ancient microorganisms.

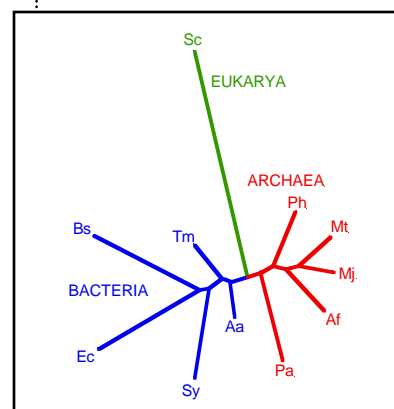
Roadmap Objectives

#4

Genomic Clues to Evolution

#5

Linking Planetary & Biological Evolution



Roadmap Objectives

#4

Genomic Clues to Evolution

#5

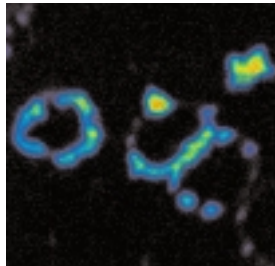
Linking Planetary & Biological Evolution

#6

Microbial Ecology

#7

Extremes of Life



The first part of this project has made great progress during the reporting period. For the first time, the ion microprobe in a multi-collection mode was used to determine the carbon isotopic composition of about 15 different microfossils specimens. Previously, we had only been able to use the mono-collection mode of the ion microprobe for this task. The multi-collection mode runs faster and has better precision. During the reporting period, these ion microprobe analyses formed the center of a paper written for *Geology*. The paper has been accepted for publication and is expected to appear in print soon. We had hoped to have this paper published sooner, but it was delayed for Dr. House to finish his Ph.D. dissertation.

The second aspect of our project has been the study of carbon isotopic fractionation in a diverse set of microorganisms. This work is proceeding well. To understand our original results better, we have further studied the carbon isotopic fractionation of the *Methanococcus* species. We expect to submit a paper soon. This project will help in the development of life detection strategies, a principle goal of astrobiology.

HIGHLIGHTS

- With this project, we have been able to study the biochemistry of 2 billion-year-old microfossils. Publication in August 2000 *Geology* also work featured in August 2000 *Science*.

Roadmap Objectives

#4

Genomic Clues to Evolution

#5

Linking Planetary & Biological Evolution

Project

Timescale for the Early Evolution of Life on Earth: Molecular Evolutionary Approach

Senior Project Investigator(s):
S. Blair Hedges

ACCOMPLISHMENTS

The overall goal of research in this lab is to develop an accurate timescale for the evolution of life on Earth, to compare it with particular aspects of the history of Earth (rise in oxygen, climate, plate tectonics, etc.). Such a synthesis will help to focus the search for extraterrestrial life. In our work, we use DNA and protein sequence data both from public databases and from sequences generated in our laboratory.

Prokaryote Evolution Study

We aligned sequences of over 300 genes from genome projects to examine the earliest divergences, and we have been conducting phylogenetic and molecular clock analyses. Because of many apparent lateral transfer events, interpretation of phylogenies and analysis requires careful study. Chen and Watanabe are involved in this work. Our results show the divergence of the last common ancestor was earlier than previously estimated, yet compatible with Earth's geologic history. This work also involved some revision of statistical methods. A manuscript is being prepared.

Year 2

Eukaryote Evolution Study

We estimated divergence times for major lineages of protists, fungi, animals, and plants. Of particular interest is the finding that fungi are much older than previously thought, with major groups originating 1.5-1.1 Ga. This suggests an early colonization of land in the Proterozoic, with implications for weathering, climate, and co-evolution with animals. Heckman, Geiser, Thompson, and Eidell are involved in this work, and a manuscript is being prepared. The relationships and timing of metazoan evolution are being studied by Lyons-Weiler, Poling, and Shoe. Van Tuinen and Pfaff are comparing phylogeny and timing of vertebrates that diverged in the Paleozoic and Mesozoic with plate tectonics and impact events. One study was published by this group, and another is being completed. Thanks to hard work of the research personnel, most supported by non-NAI funds, we have accomplished more on these projects than initially projected. During the next project year, we plan to increase our productivity by including EST (expressed sequence tags) and new genome data in the analyses to obtain more reliable time estimates for early events in the history of life. Collaborative work is planned with other members of the Evolutionary Genomics Focus Group, as well as with Japan's National Institute of Genetics (Center for Information Biology).

HIGHLIGHTS

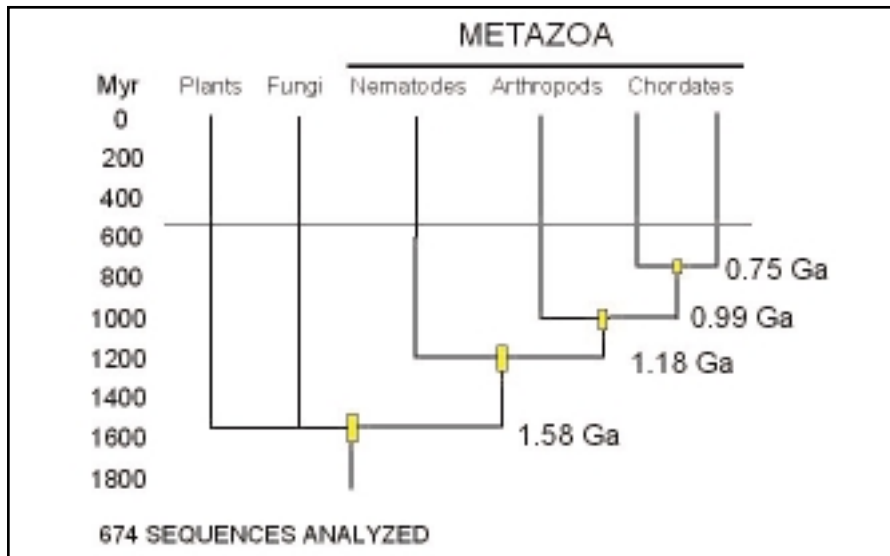
Two studies described above, now being completed, are both of general interest and have "headline" potential (topics listed below). However, I would prefer to wait until they are through the manuscript preparation and review process before releasing details.

- Prokaryote Evolution Study

Our results show a relationship between the origin of cyanobacteria and some aspects of Earth history.

- Eukaryote Evolution Study

Our finding that fungi are much older than previously thought (with major groups originating 1.5-1.1 Ga), suggests an early colonization of land in the Proterozoic, with implications for weathering, climate, and co-evolution with animals.



Roadmap Objectives

#4

Genomic Clues to
Evolution

Project

Timescale for the Early Evolution of Life on Earth: Molecular Evolutionary Approach

Senior Project Investigator(s):
Masatoshi Nei

ACCOMPLISHMENTS

The main research goal of Task III is to develop statistical methods for estimating the times of origin for the major groups of organisms using molecular data, then infer major events of early stages of evolution on Earth using a large amount of sequence data. For this purpose, it is essential to have reliable and efficient statistical methods for constructing phylogenetic trees.

At the present time, there are three major methods for constructing trees from molecular data: the maximum parsimony (MP), minimum evolution (ME), and maximum likelihood (ML) methods. In these methods, phylogenetic trees are constructed by minimizing a specific optimality score. All of these methods are very time-consuming when an extensive tree search is conducted. However, it is unclear whether extensive tree-search algorithms are really necessary for obtaining the true tree with a high probability. We therefore conducted an extensive computer simulation comparing the efficiencies of simple and extensive search algorithms in obtaining the true tree.

Our computer simulation studies comparing these algorithms show that, although extensive search algorithms always give smaller optimality scores than simple algorithms, the deviations of inferred trees from the true tree are nearly the same. This indicates that there is no need to use extensive search algorithms. Simple algorithms are sufficient for phylogenetic inference (Takahashi and Nei, 2000; Pointkivska, Takahashi, and Nei, 2000).

We also investigated statistical methods that would give reliable estimates of evolutionary times when many genes evolving with different rates are used. We have shown that it is generally better to concatenate the distances for different genes and then estimate times from concatenate distances (concatenate distance approach). This is better than estimating times by averaging the estimates obtained for individual genes (individual gene approach) (P. Xu and M. Nei, unpublished). However, the best way of weighting distances for different genes is still unclear, and we are currently investigating this problem.

Furthermore, we studied the pattern of evolution of ancient genes such as ubiquitin and histone genes and showed that these genes evolved faster than in protists than in other eukaryotes (Nei, Rogozin, and Pointkivska, 2000).

Year 2

Evolution of Atmospheric O₂, Climate, and the Terrestrial Biosphere: Approaches from Field-Oriented Geochemical Investigations

Project

Senior Project Investigator(s):
Lee R. Kump

ACCOMPLISHMENTS

Part 1

In the last year, I have focused on model development in two study areas: (1) the rise of oxygen and its relationship to mantle redox evolution (poster presented at the First Astrobiology Science Conference); and (2) the conditions favorable for development of oceanic anoxia under an oxygenated atmosphere (work with my graduate student, Roberta Hotinski) (Hotinski et al., in press; Hotinski et al., submitted).

The mantle redox modeling study will be incorporated into a manuscript being prepared for journal submission with Jim Kasting. I am proposing a new hypothesis, that the oxidation state of volcanic gases increased episodically through Earth history in response to mantle plume activity, with a major event near the Archean/Proterozoic boundary that is associated with widespread glaciation and a substantial increase in atmospheric pO₂. During the summer of 1999, samples of glaciomarine rocks (diamictites) putatively associated with this event were collected in the Meteorite Bore section of Western Australia. These glaciomarine rock samples are now being prepared for carbon isotopic analysis (in collaboration with Jay Kaufman, a PSARC affiliate at the University of Maryland). We will attempt to relate this glacial event to glaciomarine rocks Kaufman has sampled in Canada and South Africa and to the anticipated disturbance of the carbon cycle associated with the plume activity.

Finally, Andrew Kurtz (PSARC postdoc) and I have collected samples of marine cherts of all ages, so that we can attempt to reconstruct the temporal evolution of the Ge/Si ratio of the oceans. The Ge/Si ratio, like the Sr isotopic composition, reflects the interplay between hydrothermal weathering inputs to the ocean. The isotopic composition of the riverine end-member, however, reflects the intensity of chemical weathering on land. Part of our motivation, therefore, is to contrast Precambrian and Phanerozoic weathering processes, especially the relative effects of microbiota and vascular plants on the process. The ICP-MS (inductively coupled plasma-mass spectrometry) instrument has now been modified by Andy to allow Ge determination, and Paul Knauth has graciously provided us well-characterized chert samples.

This summer, we will analyze the chert samples for Ge/Si ratios and study the Australian diamictites with organic carbon isotopic analysis. Our paper on mantle redox and atmospheric oxygen will also be submitted for publication. A new field project will be undertaken with Michael Arthur to Arizona, where Paleoproterozoic pelagic marine sedimentary rocks are preserved. Our goal in this project is to assess the nature of pelagic marine biological productivity in the Paleoproterozoic ocean. This is a study complementary to one completed (and about to be submitted for publication) on the Pethei Platform of the Northwest Territories, Canada, where we found carbon isotopic evidence for an active marine planktonic biota.

Roadmap Objectives

#5

Linking Planetary &
Biological Evolution

#7

Extremes of Life

#12

Effects of Climate &
Geology on Habitability

#14

Ecosystem Response
to Rapid
Environmental Change

#15

Earth's Future
Habitability

Part 2

During the past year, Roberta Hotinski has completed her thesis work at Pennsylvania State University and submitted two of three main thesis chapters for publication.

The first of the submitted chapters is entitled "Opening Pandora's Box: Implications of Open-System Modeling for Interpretations of Anoxia." This is now in press in *Paleoceanography*. This study reveals that: (1) the short-term responses of marine phosphate and oxygen cycles captured by closed-system box models may be reversed on long time scales (>50,000 years); and (2) the open-system and vertically stratified nature of the ocean must be captured in order to assess accurately the causes of long-term anoxia using box-models of ocean biogeochemistry.

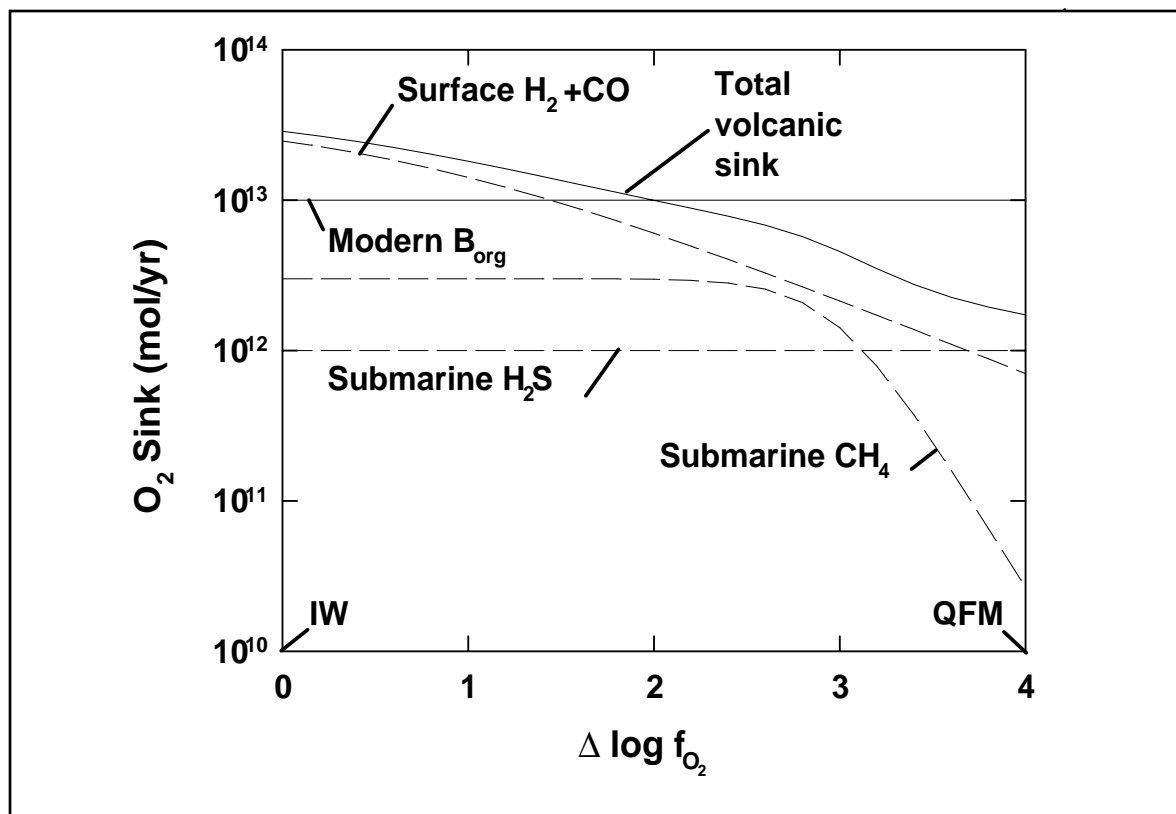
The second submitted chapter is another study of controls on deep-sea oxygenation. It is entitled "Ocean Stagnation and Permian Anoxia" and has been submitted to *Geology*. We find that high-latitude warming, roughly consistent with records of Late Permian climate, causes reduced thermohaline overturning and deepwater anoxia in a simulated Permian ocean. This result supports previous suggestions that anoxia may have played a role in the End-Permian extinction.

In the next few months, Hotinski will be preparing the third and final chapter of her thesis for publication. This chapter, entitled "Interpretation of a Neoproterozoic $\Delta^{13}\text{C}$ gradient from the Pethei Platform (Great Slave Lake Supergroup, Northwest Territories)" presents the results of a carbon isotope study of a 1.9 Ga stromatolitic reef in Canada. We find only a small vertical gradient (about -0.5‰) between $\Delta^{13}\text{C}$ of marine cements formed in shallow environments and cements in basinal carbonates inferred to have been deposited at ~1 km depth. We attribute the small gradient to high marine DIC (dissolved inorganic carbon) rather than to weak biological pumping.

In addition to completing this third publication, Roberta Hotinski is continuing to work with Lee Kump over the summer to improve our biogeochemical general circulation model for work on astrobiological problems.

Part 3

Greg Retallack (University of Oregon) has been diverted by funding for a variety of projects not relevant to PSARC, but he continues studies of Precambrian fossils. He reported on a new occurrence of Ediacara-like fossils from Montana at the *Geological Society of America Meeting* this past November 1999. A manuscript was also completed with Doug Elmore, Monika Cogoini, and Nathan Sheldon on using paleomagnetic susceptibility anomalies to recognize paleosols in late Precambrian fluvial successions. Work continues on small lichen-like fossils in the Waterval Onder paleosols (2.2 Ga). CAT-scanning (computerized axial tomography-scanning) of these fossils at the University of Texas has finally provided good images. A new edition of the textbook, *Soils of the Past*, was sent to Blackwell Science Publishers. This textbook includes much of interest to PSARC, including the currently compelling case, from isotopic studies, for life in soils well back into the Precambrian.



HIGHLIGHTS

Field Expeditions

Hamersley and Pilbara District, Western Australia

Date: July 26 – August 18, 1999. Participants: Leader: H. Ohmoto. Pennsylvania State University: L. Kump, M. Bau, Y. Watanabe, K. Yamaguchi, S. Ono, J. Eigenbrode, and C. Brachle. PSARC Associates: M. Nedachi, K. Hayashi, T. Kakegawa, H. Naraoka (Tokyo Metropolitan University), M. Barley, N. Beukes (Rand Afrikaans University). Kagoshima University: Y. Nedachi, M. Hoashi, and J. Nozaki. Tohoku University: M. Matsunodaira. University of Western Australia: B. Kupetz.

Samples of glaciomarine rocks (diamictites) were collected in the Meteorite Bore section of Western Australia. These glaciomarine rocks are putatively associated with a major event, an increase in the oxidation state of volcanic gases near the Archean/Proterozoic boundary, which is associated with widespread glaciation and a substantial increase in atmospheric pO_2 , all related to mantle redox evolution.

Roadmap Objectives

#1

Sources of Organics on Earth

#4

Genomic Clues to Evolution

#5

Linking Planetary & Biological Evolution

#15

Earth's Future Habitability

Project

Evolution of Atmospheric O₂, Climate, and the Terrestrial Biosphere: Approaches from Field-Oriented Geochemical Investigations.

Senior Project Investigator(s):
Hiroshi Ohmoto

ACCOMPLISHMENTS

In addition to overseeing the entire activities of the Penn State Astrobiology Research Center (PSARC) as the Director, Hiroshi Ohmoto supervises the research projects carried out by Michael Bau (Research Associate), Kosei Yamaguchi (Ph.D. candidate), Shuhei Ono (Ph. D. candidate), Yumiko Watanabe (Ph.D. candidate), Robin Mock (Ph.D. candidate), and John Rimmer (senior, undergraduate). The progress made in these research projects is reported separately by each of these investigators.

Hiroshi Ohmoto has also been involved in the following research projects:

- Paleosols

In collaboration with Professor Nic Beukes (PSARC Associate Member and Rand Afrikaans University), Professor Munetomo Nedachi (PSARC Associate Member and Kagoshima University), and their students, I have been carrying out detailed geochemical investigations on ~2.3 Ga paleosols in South Africa, 2.4 Ga paleosols in Ontario, and ~2.9 Ga paleosols in Australia. (See Highlights section below.).

- Pyrites in Shales

In collaboration with Professor Takeshi Kakegawa (PSARC Associate Member and Tohoku University), I have been carrying out a sulfur isotopic investigation of pyrite crystals in Archean and Proterozoic shales. Some of the results are published in *Precambrian Research* 96: 209-224 (1999).

- Siderite in Archean Sediments

The significance of siderite in some Archean sediments in terms of the atmospheric evolution is discussed in *Geology* 27: 1151-1152 (1999).

All the research projects described above are the field-oriented geochemical investigations that are aimed to constrain the levels of atmospheric O₂ and its connection to the evolution of life on early Earth. Investigation results obtained to date support one of the two working hypotheses presented in our initial NAI proposal: Development of an oxygenated atmosphere and divergence of organisms occurred prior to 3.5 billion years ago. The field-oriented research will be continued to examine the two hypotheses critically and to develop an accurate model for the evolution of environment and organisms.

To understand the mechanisms of regulating the atmospheric oxygen level through geologic time, Professor Antonio Lasaga (Yale University) and I have been modeling the dynamics of oxygen and carbon cycles that incorporate the kinetics of redox reactions during weathering. Our preliminary results suggest the importance of soil weathering

in regulating the atmospheric oxygen level. Next year, we will continue modeling evolution of the atmospheric pO_2 and pCO_2 under a variety of scenarios.

HIGHLIGHTS

• Discovery of ~2.3 Billion-Year-Old Laterites

Modern laterites, soils highly enriched in ferric iron (Fe_3+), are products of intense sub-aerial weathering of rocks, mostly in the tropics. Laterite formation requires: (1) an abundance of organic acids (generated from decay of surface vegetation and soil organisms) to mobilize both ferrous (Fe_2+) and ferric iron in rocks and laterites; and (2) the abundance of molecular oxygen to precipitate the mobilized iron as ferric-hydroxides.

Through collaborative research with Professor Nic Beukes (Rand Afrikaans University), we have recently discovered paleolaterites that formed over a >300,000 km² area in South Africa and Botswana about 2.3 billion years ago. This recognition strongly supports a theory that the oxygenated atmosphere and the biomass on land developed more than 2.2 billion years ago. This discovery was reported by Ohmoto et al. at the *Annual Meeting of the Geological Society of America* (October, 1999), and a manuscript for submission to *Science* is being prepared.

Field Expeditions

Several field expeditions were carried out (during May, 1999 – August, 1999) to observe the field occurrences of a variety of rocks formed 3.5-2.0 Ga (billion years) ago. We systematically collected these samples for a variety of geochemical investigations aimed at understanding the connection between the evolution of environment (e.g., O_2 and CO_2 contents of the atmosphere and ocean) and the evolution of biota in the oceans and lands in the early history of Earth.

Our investigations are especially focused on the following rock types: paleosols, uraniferous quartz-pebble conglomerates, red beds, (bio)chemical sediments (banded iron formations, carbonates, cherts, stromatolites), and shales. Hiroshi Ohmoto of Pennsylvania State University was Leader of the field expeditions outlined below.

• *The Elliot Lake District, Ontario, Canada*

Date: May 23 – May 26, 1999. Participants: H. Ohmoto (Leader), L. Kump, J. Kasting, M. Hurtgen, R. Hotinski, A. Ennyu, J. Lamberski, S. Ono, Y. Watanabe, M. Bau, C. Brachle, A. Pavlov, and K. Yamaguchi (all from Pennsylvania State University); G. Bennett of the Ontario Geological Survey (Canada)

• *The Thunder Bay and Steep Rock Districts, Ontario; the Val D'Or District, Quebec; the Abitibi District, Ontario, Canada*

Date: May 26 – June 7, 1999. Participants: H. Ohmoto (Leader), M. Bau, Y. Watanabe, S. Ono, and K. Yamaguchi (all from Pennsylvania State University)

• *Hamersley and Pilbara District, Western Australia*

Date: July 26 – August 18, 1999.

Participants: H. Ohmoto (Leader), with these investigators and affiliations: Pennsylvania State University: L. Kump, M. Bau, Y. Watanabe, K. Yamaguchi, S. Ono, J. Eigenbrode,

Roadmap Objectives

#1

Sources of Organics on Earth

#4

Genomic Clues to Evolution

#5

Linking Planetary & Biological Evolution

#15

Earth's Future Habitability

and C. Brachle; PSARC Associates: M. Nedachi, K. Hayashi, T. Kakegawa, H. Naraoka (Tokyo Metropolitan University), M. Barley, N. Beukes (Rand Afrikaans University); Kagoshima University: Y. Nedachi, M. Hoashi, and J. Nozaki; Tohoku University: M. Matsunodaira; University of Western Australia: B. Krapez

Field Expedition in 2000

Location: *Hokkalampi District, Finland*

Date: June 5 – June 23, 2000

Participants: H. Ohmoto (Leader) and Y. Watanabe from Pennsylvania State University; S. Stafford (University of Pittsburgh); N. Watanabe (Niigata University), J. Marmo and J. Karhu (University of Finland)

Objectives: (1) To examine the field occurrences of ~2.4 Ga paleosols, ~2.7 Ga banded iron formations, and ~2.2 Ga carbonates; and (2) to collect samples systematically for geochemical investigations.

Project

Evolution of Atmospheric O₂, Climate, and the Terrestrial Biosphere: Approaches from Field-Oriented Geochemical Investigations.

Senior Project Investigator(s):
Hiroshi Ohmoto

ACCOMPLISHMENTS

Task IV of the Penn State Astrobiology Research Center (PSARC) aims to determine the connections between the origin and evolution of organisms and the evolution of atmospheric oxygen, carbon dioxide, and climate. As a member of the PSARC team, Kosei Yamaguchi's research goal has been to constrain the evolution of atmospheric oxygen through geochemical investigations of sedimentary rocks that are 3.9-0.7 Ga in age.

The first part of our research focuses on shales that are 3.4-1.9 Ga in age. A large number of shale rock samples were collected from drill cores in South Africa (1996 & 1998) and in Australia (1999). So far, approximately 150 samples from several drill holes have been powdered, then decomposed, using two methods (alkali fusion and mixed acid digestion). These samples were then analyzed for the concentrations of ~60 elements. These included major, minor, and trace elements including REEs (rare earth elements), utilizing ICP-AES (inductively coupled plasma-atomic emission spectroscopy) and ICP-MS (inductively coupled plasma-mass spectroscopy) methods. Ferric/ferrous ratios of the samples were determined by titration, and the C/H/N/S contents were determined by an elemental analyzer. Carbon and sulfur isotopic compositions of organic matter and pyrite in shales were also determined. These data are now being interpreted and will be published in a few papers.

The second part of our research focuses on rare earth element geochemistry of Precambrian banded iron formations (BIFs). Through a detailed analysis of all the data published in the literature, we hope to understand the following major questions: (1) the extent and variation of hydrothermal fluid involvement in the formation of BIFs;

(2) the redox states of sea water in the depositional basins of BIFs; and (3) the relationships between the BIF types (e.g., oxide- or carbonate-BIFs vs. sulfide BIFs; Fe-rich vs. Mn-rich BIFs); and (4) relationships of BIF types with the conditions stated in (1) and (2) above. Some of our major findings include the relationship between Ce and Eu anomalies. Additionally, we used a new method to calculate Ce anomalies to exclude the effect of La anomalies. Part of this research was presented at the *First Astrobiology Science Conference* at NASA Ames Research Center in April 2000.

HIGHLIGHTS

- For background perspective about our research, many previous investigators have recognized the presence of negative Ce anomalies in Precambrian BIFs. If the negative Ce anomalies are real, it implies that the oceans were (at least partially) oxygenated.
- We (Yamaguchi and Ohmoto) have carried out a detailed examination of all the published data on BIFs of Precambrian age, using a new method to define true Ce anomalies. (Cherts and carbonates will also be examined.) The results of our study indicate that most of the previously suggested "negative Ce anomalies" are artifacts of positive La anomalies.
- While many samples are found to show virtually no Ce anomalies, some samples (both pre- and post-2.2 Ga in age) are found to show either "positive" or "negative" Ce anomalies. Such data are consistent with a model of BIF deposition in locally anoxic basins that were overlain by a body of oxygenated seawater.

Field Expeditions

- *The Elliot Lake District, Ontario, Canada*

Date: May 23 – May 26, 1999.

Participants: H. Ohmoto (Leader), L. Kump, J. Kasting, M. Hurtgen, R. Hotinski, A. Ennyu, J. Lamberski, S. Ono, Y. Watanabe, M. Bau, C. Brachle, A. Pavlov, and K. Yamaguchi (all from Pennsylvania State University); G. Bennett of the Ontario Geological Survey (Canada)

- *The Thunder Bay and Steep Rock Districts, Ontario; the Val D'Or District, Quebec; the Abitibi District, Ontario, Canada*

Date: May 26 – June 7, 1999.

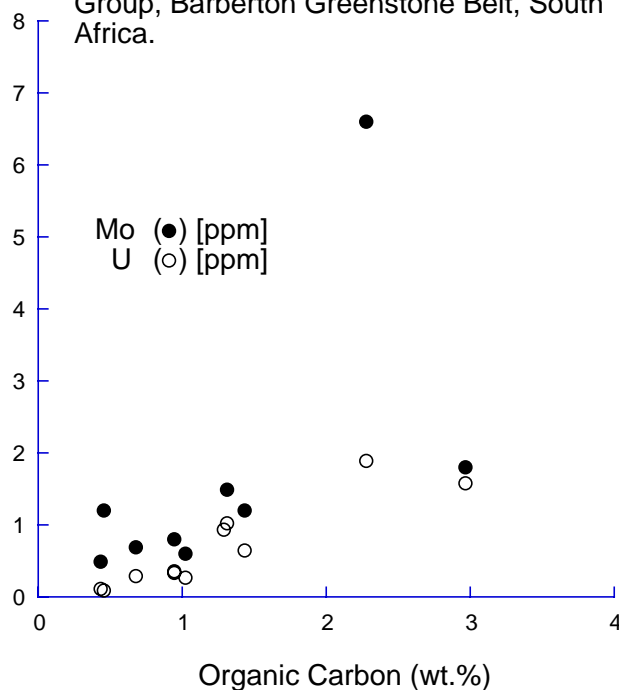
Participants: H. Ohmoto (Leader), M. Bau, Y. Watanabe, S. Ono, and K. Yamaguchi (all from Pennsylvania State University)

- *Hamersley and Pilbara District, Western Australia*

Date: July 26 – August 18, 1999.

Participants: H. Ohmoto (Leader), with these investigators and affiliations: Pennsylvania

Relationship between Mo and U contents (ppm) and organic carbon contents (wt.%) of black shales from the ~3.4 Ga Fig Tree Group, Barberton Greenstone Belt, South Africa.



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State University: L. Kump, M. Bau, Y. Watanabe, K. Yamaguchi, S. Ono, J. Eigenbrode, and C. Brachle; PSARC Associates: M. Nedachi, K. Hayashi, T. Kakegawa, H. Naraoka (Tokyo Metropolitan University), M. Barley, N. Beukes (Rand Afrikaans University); Kagoshima University: Y. Nedachi, M. Hoashi, and J. Nozaki; Tohoku University: M. Matsunodaira; University of Western Australia: B. Krapez

Project

Evolution of Atmospheric O₂, Climate, and the Terrestrial Biosphere: Approaches from Field-Oriented Geochemical Investigations.

Senior Project Investigator(s):
Hiroshi Ohmoto

ACCOMPLISHMENTS

Oxygen and lead isotopic compositions of individual uraninite from Uranium ore from Elliot Lake district (Canada) were measured and interpreted, together with the large scale history of the hosting Huronian Basin. The manuscript has been prepared for publication.

Shuhei Ono made a detailed compilation of literature data on the dissolution rates of uraninite and UO₂ spent nuclear fuel. The recent well-controlled experiments show slower dissolution rates than those previously reported by ~2 orders of magnitude. Therefore, the survival of detrital uraninite is not a function of oxygen level. Detrital uraninite survival (as a function of oxygen level) has been previously used as evidence for an anoxic atmosphere before 2.2 Ga. Our research finds that the lower oxygen limit given by survival of uraninite is as high as 100 PAL. This result was presented at the *First Astrobiology Science Conference* in April 2000.

These results suggest that it is very important to study the dissolution rate of "natural" uraninite under both low and high oxygen environments. New experiments to study the dissolution rate at various oxygen and CO₂ levels are being set up at this time.

HIGHLIGHTS

- Constraints on the oxygen level of the Archean atmosphere based on new data on dissolution rates of uraninite
- Survival of detrital uraninite is not a function of oxygen level, and this has been previously used as evidence for an anoxic atmosphere before 2.2 Ga.

Field Expeditions

- *The Elliot Lake District, Ontario, Canada*

Date: May 23 – May 26, 1999.

Participants: H. Ohmoto (Leader), L. Kump, J. Kasting, M. Hurtgen, R. Hotinski, A. Ennyu, J. Lamberski, S. Ono, Y. Watanabe, M. Bau, C. Brachle, A. Pavlov, and K. Yamaguchi (all from Pennsylvania State University); G. Bennett of the Ontario Geological Survey (Canada)

Year 2

- *The Thunder Bay and Steep Rock Districts, Ontario; the Val D' Or District, Quebec; the Abitibi District, Ontario, Canada*

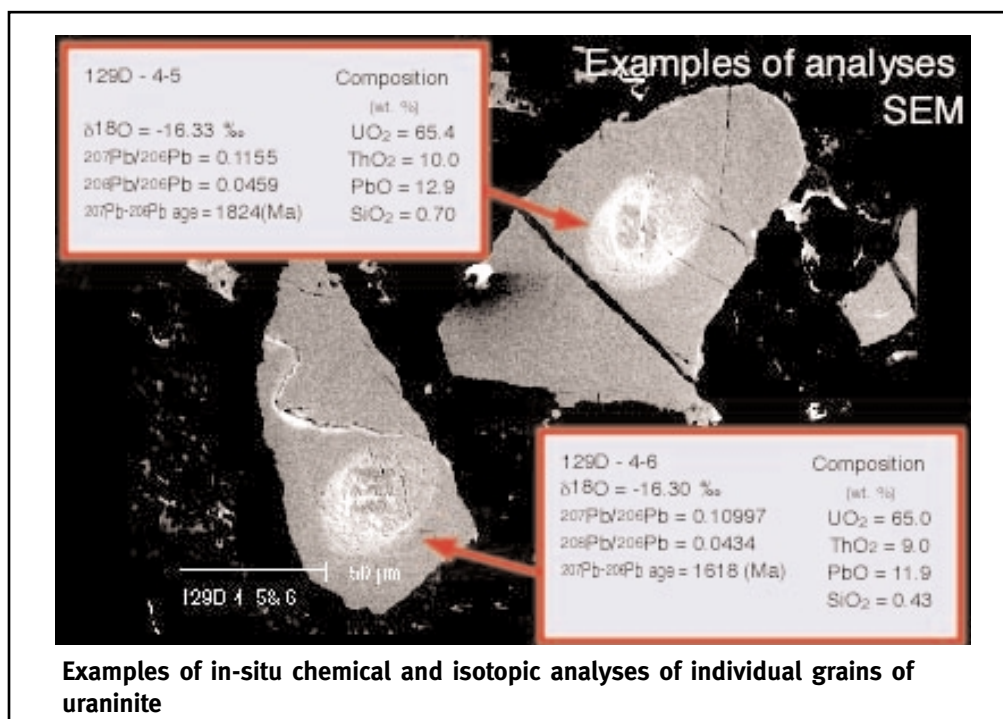
Date: May 26 – June 7, 1999.

Participants: H. Ohmoto (Leader), M. Bau, Y. Watanabe, S. Ono, and K. Yamaguchi (all from Pennsylvania State University)

- *Hamersley and Pilbara District, Western Australia*

Date: July 26 – August 18, 1999.

Participants: H. Ohmoto (Leader), with these investigators and affiliations: Pennsylvania State University: L. Kump, M. Bau, Y. Watanabe, K. Yamaguchi, S. Ono, J. Eigenbrode, and C. Brachle; PSARC Associates: M. Nedachi, K. Hayashi, T. Kakegawa, H. Naraoka (Tokyo Metropolitan University), M. Barley, N. Beukes (Rand Afrikaans University); Kagoshima University: Y. Nedachi, M. Hoashi, and J. Nozaki; Tohoku University: M. Matsunodaira; University of Western Australia: B. Krapez



Evolution of Atmospheric O₂, Climate, and the Terrestrial Biosphere: Approaches from Field-Oriented Geochemical Investigations

Project

Senior Project Investigator(s):
Katherine Freeman

ACCOMPLISHMENTS

Our research focuses on unraveling the biogeochemical record of carbon and oxygen cycling in the late Archean period by determining relationships between physical envi-

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ronments and biological sources of preserved organic matter. We are testing the hypothesis that depositional environments are linked to microbial ecosystems through the creation and stabilization of oxygen microenvironments or stratified water columns. To do this research, we are integrating stratigraphic and organic geochemical studies. Beginning in June, partial funding for this project from the National Science Foundation to Kate Freeman will cover further fieldwork and lab analyses.

Sample preparation of rock samples collected last August is nearly complete. Analyses for organic carbon and elemental (CHNS) abundances in both bulk and kerogen samples are underway. Samples are currently being prepared for kerogen hydropyrolysis.

Plans for the upcoming year include 6 weeks of fieldwork (mid-June to mid-August) in the Hamersley Basin (Western Australia), in which additional cores and outcrops will be stratigraphically logged and sampled for analyses. Stratigraphic sections and carbonate carbon isotopes will be used to clarify correlations in the larger stratigraphic database developed by Dr. Mark Barley and Dr. Brian Krapez (University of Western Australia). Petrographic analyses to further define specific lithofacies will commence in the Fall 2000 and is targeted for completion in the Spring 2001.

Biomarker analyses and a test for contamination are integral parts of our study and involve detailed investigation of both extractable organic matter and non-extractable kerogen. Molecular analysis of trace components in the extracts of core samples will be conducted in October & November 2000 at Dr. Roger Summons' laboratory in collaboration with Jochen Brocks. Major component molecular analysis will start in the Spring 2001 at Pennsylvania State University, in addition to analysis of kerogen hydropyrolysates. Molecular and kerogen analyses will be spread over the next two years. Samples recently collected will be processed in the same manner as the first set and analyzed for bulk and kerogen properties by Summer 2001.

HIGHLIGHTS

Field Expeditions

- August 1999, *Hamersley Basin, Western Australia*

Samples were collected from core and a single outcrop for organic, geochemical, and sedimentary studies. This trip was led by Dr. Hiroshi Ohmoto (Pennsylvania State University) and guided by Dr. Mark Barley and Dr. Brian Krapez (University of Western Australia).

- June-August 2000, *Hamersley Basin, Western Australia*

Samples will be collected across the basin in order to establish organic geochemical relationships to depositional environments and/or temporal and geographic variability. Dr. Mark Barley and Dr. Brian Krapez (University of Western Australia) are assisting with logistics and are providing guidance in determining the best field sites to sample and log. Matt Hurtgen (astrobiology graduate student at Pennsylvania State University) will assist in fieldwork.

Year 2

Evolution of Atmospheric O₂, Climate, and the Terrestrial Biosphere: Approaches from Field-Oriented Geochemical Investigations

Project

Senior Project Investigator(s):
Jennifer Bland

ACCOMPLISHMENTS

Our research purpose is to identify and characterize the carbon sources for bacterial populations in soils overlying methane seeps. In particular, we seek to identify potential sources of nitrous oxide and sinks for methane.

Autotrophic nitrifiers utilize ammonium as an energy source, oxidizing it to nitrous oxide. Methanotrophs use methane as a source of carbon and energy, oxidizing it to carbon dioxide. Methane and ammonium molecules are similar in size, shape, and geometry. There is also similarity in the oxidizing enzyme used by the autotrophic nitrifiers and methanotrophs. Because of these similarities, both methane and ammonium are used by both groups of bacteria for energy. Studies directed by Mandernack suggest methanotrophs are an important source of nitrous oxide, and autotrophic nitrifiers are potentially important methane consumers.

We are examining the bacteria populations by using phospholipid fatty acids which are diagnostic for individual taxonomic groups. The $\Delta^{13}\text{C}$ values of bacterial lipid biomarkers provide insight to their carbon sources and metabolic pathways. Of a total of eighteen soil and culture samples, phospholipids from twelve have been extracted and analyzed using gas chromatography. Five of the extracted samples are microbial cultures of the methanotroph *M. methanicus*. The remaining seven extracted samples are from soil in sites with elevated methane flux, including a landfill and a thermogenic methane seep.

HIGHLIGHTS

Field Expeditions

In August of 1999, Kevin Mandernack from the Colorado School of Mines collected soil samples from a thermogenic methane seep. The samples were collected from the Pine River site, which is about 20-25 miles east-northeast of Durango, Colorado.

Neoproterozoic Variations in Carbon and Sulfur Cycling

Project

Senior Project Investigator(s):
Michael A. Arthur

ACCOMPLISHMENTS

Efforts have been made to reconstruct the detailed history of climate, atmospheric chemistry (pO₂ and pCO₂), ocean chemistry, and the global cycles of carbon and sulfur

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during the 800-500 Ma period. These endeavors have involved modeling as well as geochemical analyses of Neoproterozoic sequences in Namibia and Australia. For much of the late Neoproterozoic, we have generated a continuous record of sulfur isotopic composition for marine sulfate from Namibian strata. We have also produced geochemical cycle models that successfully produce stable isotope variations observed in the records.

Our objectives for the next year are to complete our work on Namibian Neoproterozoic sulfur isotopes and submit a manuscript on that work to *Nature* or other journal. We will complete analysis of the Neoproterozoic samples from South Australia (collected in Fall 1999), in order to have significant temporal overlap in our S isotope secular curve for comparison to Namibia. This will lead to a longer second manuscript that discusses the global nature of the isotope variations, relating this to sulfide sulfur isotopes as well.

In this next year, we will conduct field work in the Neoproterozoic of the Great Basin. This will probably occur late this Fall 2000 and will constitute the third and last set of sections to be analyzed for the late Neoproterozoic story. M. Hurtgen and M. Arthur will be in the field together to work with Bruce Runnegar (UCLA) and possibly Tony Prave (University of St. Andrews, Scotland). We will probably be out there either just before or after the *Geological Society of America Meeting* (Reno, Fall 2000). Lee Kump and M. Arthur plan to spend about a week sampling "deepwater" sediments associated with Paleoproterozoic ophiolites in Arizona as well.

Field work will also be conducted in the Hammersley Basin (about 1 month or so) for the late Archean and Paleoproterozoic. This is an opportunity for M. Hurtgen to examine sulfate sulfur isotopes in carbonates from this sequence (some in association with iron formations) at no major cost to PSARC.

HIGHLIGHTS

- Our carbon cycle and climate modeling (Arthur & Kump) has yielded insights into the causes of global glaciations ("Snowball Earth") in the Neoproterozoic and explains the patterns in oceanic carbon isotope variations prior to "Snowball" events. Although enhanced organic carbon preservation in an anaerobic ocean initially drives atmospheric $p\text{CO}_2$ down and causes sea ice growth at high latitudes, the carbon isotope patterns suggest that a different mechanism takes over once the globe is about half covered with ice. An enhanced hydrologic cycle in low latitudes, plus the low-latitude position of most continental masses enhances the global silicate weathering rate and draws down $p\text{CO}_2$ to the threshold necessary for global ice cover to develop.
- We have generated the first "high" resolution sulfate sulfur isotope record for the Neoproterozoic using a technique developed in our laboratory. Our sulfur isotope studies of the late Neoproterozoic (Hurtgen, Arthur, & Suits) have discovered major variations in the sulfur isotopic composition of seawater sulfate. The amplitude and pattern of these variations suggest that oceanic sulfate concentrations in the late Neoproterozoic were low, in comparison to the present. In addition, the pattern of sulfur isotope variation associated with "Snowball" events supports the scenario for these global glaciations, as developed by Paul Hoffman and colleagues at Harvard University.

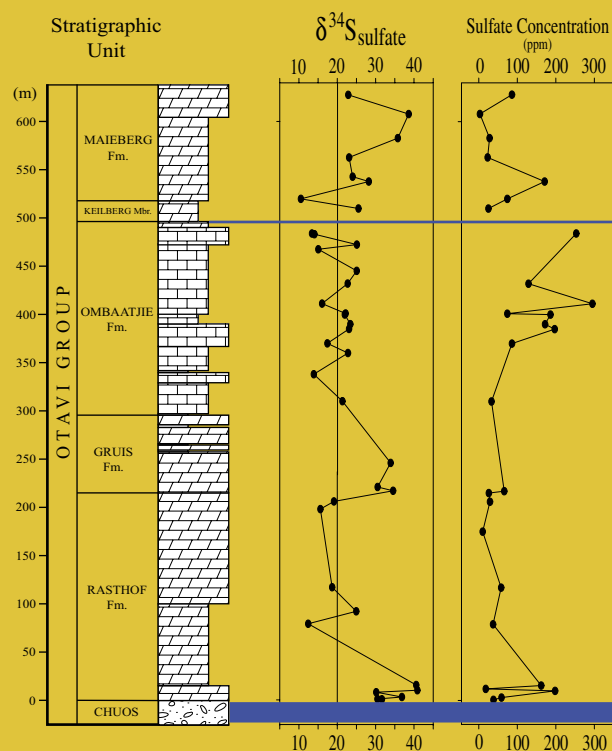
Field Expeditions

Location: *South Australia (Adelaide Geosyncline)*

When: September, 1999 (2 weeks)

Field Party: Michael Arthur and Matthew Hurtgen (PennState University), Tracy Frank (Queensland University, Brisbane) Purpose: Sample Neoproterozoic carbonates for sulfur and carbon isotope work. Dr. Frank assisted in the field. The research is a part of Matt Hurtgen's Ph.D dissertation research.

Namibian Carbonates (Neoproterozoic)



Hurtgen, Arthur and Suits (unpubl. data)

Sulfur isotope values for sulfate from the carbonate lattice and sulfate concentrations from a Namibian Neoproterozoic carbonate sequence (Hurtgen, Arthur, and Suits, in prep.). Three substantial positive sulfur isotope excursions occur in the record, which span perhaps 150 million years. Two of the three positive sulfur isotope excursions occur in carbonate ("caps") strata overlying glacial intervals and may reflect isotope enrichment of oceanic sulfate in anoxic oceans resulting from massive sulfate reduction during "Snowball Earth" events. The Gruis excursion may indicate a third glacial interval that did not leave a record of tillites at this locale.

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Project

Causes and Consequences of the Diversification and Extinction of Metazoans: Field, Geochemical, and Paleontological Investigations

Senior Project Investigator(s):
Mark E. Patzkowsky

ACCOMPLISHMENTS

We have begun to collect a multifaceted database of Neoproterozoic and Cambrian macrofossil occurrences. In addition to genus and species occurrences, data recorded include latitude and longitude, primary lithology, depositional environment, tectonic setting, and taphonomy of deposit. These data will permit us to evaluate the environmental and geographic context of the origin and diversification of multicellular animal communities. The data are part of a larger project on the Phanerozoic fossil record sponsored by the National Center for Ecological Analysis and Synthesis (NCEAS) in Santa Barbara. We have spent much of the past year setting up the database structure and making preliminary analyses of the data. A first manuscript was recently submitted to *Science*.

We have achieved the main goal of year 2, which was to begin collecting and entering Neoproterozoic and Cambrian macrofossil occurrences into a flexible database structure. We plan to continue collecting data over the next year. Based on the rate at which data are collected and entered into the database, I suspect that we will be ready to perform some preliminary analyses by the end of the year. I will also be directing an M.S. student on a project concerning the Late Ordovician mass extinction and its effects on marine community structure. Since this mass extinction is known to be associated with a rapid and severe glaciation event, it has important implications for Neoproterozoic Snowball Earth scenarios and in particular how global ecosystems respond to such events.

Project

Evolution of Atmospheric O₂, Climate, and the Terrestrial Biosphere: Causes and Consequences of the Diversification and Extinction of Metazoans

Senior Project Investigator(s):
Rosemary C. Capo, Brian W. Stewart

ACCOMPLISHMENTS

Paleosols as indicators of atmospheric composition
(Capo, Stewart, Stafford, Chadwick, Whipkey, Reynolds, Macpherson, Ohmoto)

Paleosols (preserved ancient soils) can preserve a record of past atmospheric chemistry, although the evidence is often complicated by later diagenetic and metamorphic events. Our efforts focus on characterization of both weathering-induced changes in bulk chemistry and distribution of trace elements among pedogenic and post-pedogenic phases in paleosols.

Preliminary results from a >2.7 Ga paleosol developed on tonalite from the Steep Rock

Year 2

Group, Superior Province, Canada shows geochemical signatures consistent with either formation in a reducing environment or formation under oxidizing conditions followed by hydrothermal alteration. Initial laser ablation-ICP-MS experiments by Associate Member Gwen Macpherson show distinctive rare earth element patterns from coexisting carbonate, Fe-rich matrix materials, and silicate minerals. Ph.D. student Sherry Stafford has also initiated a geochemical study of Archean paleosols from the Hokkalampi area, Finland.

For comparison, we are also investigating the geochemical and textural evidence for atmospheric conditions provided by Paleozoic and Quaternary soils formed under variable redox conditions. Former Ph.D. student Charles Whipkey, in association with Associate member Oliver Chadwick, examined how external inputs from seawater and precipitation affected the composition of Quaternary soils on the island of Hawaii. M.S. student Amanda Reynolds is completing a study of Pennsylvanian paleosols.

Paleoenvironmental Determination: Precambrian and Modern Carbonates
Eastern Transvaal district, South Africa (Stewart, Capo, Watanabe, Ohmoto).

A 2.6 Ga carbonate-rich sequence at the Schagen locality contains evidence of photoautotrophic organisms that may have lived in a terrestrial playa/paleosol environment. Strontium isotope results from sequential leaching experiments are consistent with multiple stages of carbonate formation in a shallow freshwater depositional environment, with variable interaction between surface-derived waters and ultramafic host rock. Our data suggest that the C isotope signatures measured from this paleosol section represent primary signatures.

Paleoproterozoic Marine Carbonates (Stewart, Capo, Bau).

Early Proterozoic marine carbonates can potentially provide important constraints on ancient oceanic element transport and oxidation state. We have begun a Sr-Nd isotopic study of Paleoproterozoic sequences from Australia and South Africa.

Initial results suggest that original marine $^{87}\text{Sr}/^{86}\text{Sr}$ ratios are preserved in several of the samples. We found ratio values as low as 0.7041 in 2.5-2.6 Ga Hammersly carbonates. In 2.4 Ga Moodri carbonates, we found $^{87}\text{Sr}/^{86}\text{Sr}$ ratios to be 0.7051. The Nd isotope work in progress will be one of the first to examine $^{143}\text{Nd}/^{144}\text{Nd}$ of Precambrian carbonate sediments.

Nama Group, Namibia (Stewart, Capo, Ono, Ohmoto).

Limestones from the Nama group span the important period in Earth's history when shelled organisms first appeared. We show that microdrilled and whole rock samples of ~0.6 Ga sedimentary rocks from the Zebra River Member of the Zaris Formation (ZR Series) fall within the range of Vendian-Cambrian marine $^{87}\text{Sr}/^{86}\text{Sr}$ values. Our data from the shelly fossil containing Kuibis Subgroup at Hauchabfontain (HF Series) are suggestive of a freshwater incursion.

We are currently attempting to distinguish between isotopic effects of deposition in lacustrine systems and overprinting of marine limestones by meteoric waters. Either of these conditions may have affected ancient carbonates.

Modern Terrestrial Carbonates (Stewart, Capo, Chadwick, Pretti, Minervini, Reynolds).

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We are conducting isotopic studies of modern lacustrine analogues. These include Pleistocene-Holocene lake sediments from Searles and Owens Lake (California) and soil carbonate in continental and tropical environments (Hawaii and New Mexico). Our goal is to identify a means of distinguishing between marine, lacustrine, pedogenic, and diagenetically altered carbonate, with the hope of better characterizing ancient carbonate depositional environments. Initial work has focused on relating modern-day cation fluxes to the strontium isotopic evolution of lacustrine and pedogenic systems.

HIGHLIGHTS

May-June, 1999

Co-I's Capo, Stewart and Ph.D. student Sherry Stafford (U. Pittsburgh) accompanied a team from Pennsylvania State University to investigate and sample Precambrian sediments and paleosols from the Elliot Lake, Thunder Bay, Steep Rock and Val D'Or Districts, Canada

May-June, 2000

Capo, Stewart and M.S. student Joseph Minervini (U. Pittsburgh) conducted field work in Owens Valley, California and the Desert Project area, New Mexico to understand the formation of terrestrial carbonates, both lacustrine and pedogenic.

June, 2000

Sherry Stafford accompanied a team from Pennsylvania State University headed by PI H. Ohmoto on a field expedition to Finland. The group investigated field relationships and conducted detailed sampling of the Hokkalampi paleosol sequence.

July, 2000

M.S. student Amanda Reynolds (U. Pittsburgh) carried out field work to sample late Paleozoic paleosols formed under reducing and oxidizing conditions associated with a large freshwater lake in Pennsylvania and West Virginia.

PUBLICATIONS & ABSTRACTS

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PROJECT TITLE: *The Environment of Prebiotic Earth: Theoretical Approach*

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- Pavlov, A.A., Kasting, J.F., Brown, L.L., Rages, K.A., & Freedman, R. (2000). Greenhouse warming by CH₄ in the atmosphere of early Earth. *Journal of Geophysical Research: Planets*, 105(5): 11,981-11,990.

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- Sheridan, P.P., & Brenchley, J.E. (2000). Characterization of a salt-tolerant family 42 b-galactosidase from a psychrophilic Antarctic *Planococcus* isolate. *Applied and Environmental Microbiology*, 66(6): 2438-2444.

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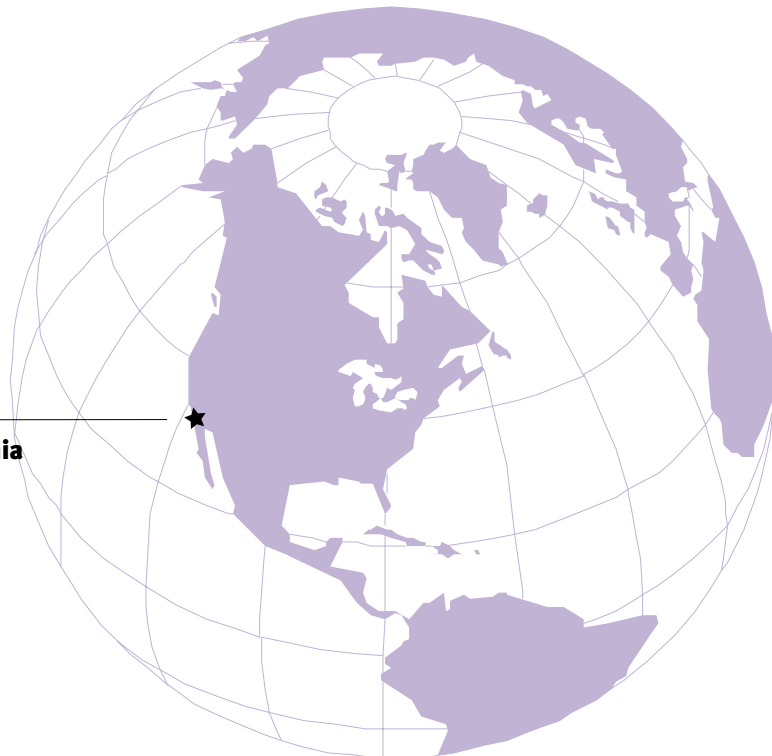
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SCRIPPS RESEARCH INSTITUTE

La Jolla, California



PRINCIPAL INVESTIGATOR



**M. Reza
Ghadiri**

SRI TEAM MEMBERS

Steve Benner,
University of Florida

Ronald Castellano,
Scripps Research Institute

A. Chavoshi,
Scripps Research Institute

Michael Churchill,
Scripps Research Institute

Stephen Craig,
Scripps Research Institute

Milan Crnogorac,
Scripps Research Institute

Andrew Ellington,
University of Texas at Austin

M. Reza Ghadiri,
Scripps Research Institute

V. Haridas,
Scripps Research Institute

Steffi Kerner,
Scripps Research Institute

P. Lopez-Deber,
Scripps Research Institute

PROPOSAL EXECUTIVE SUMMARY (1998)

What constitutes life and how living systems may be identified are perhaps the most fundamental problems in astrobiology. Under the auspices of the Scripps Research Institute, a 'virtual institute' has been assembled to explore an experimental approach to self-replication and Darwinian chemistry, the most important hallmark of life. By comparing and contrasting the results of a diverse but complementary set of experiments, we suggest that it should be possible to garner a better understanding of life and its origins.

Experimentally, Rebek will determine how replication can be encoded in molecular structure using calixerene ureas. Ghadiri and von Kiedrowski will study the kinetics of replication of encoded molecular structures such as polypeptides and DNA oligonucleotides, while Ellington will study the emergence of self-replicating nucleic acids from random sequence populations. These experiments should provide key insights into how replicating molecules may have arisen in the terrestrial prebiotic soup. Following up on the astounding discovery that peptides can self-replicate, Ghadiri will address the next level in the organizational hierarchy of living systems, i.e., the self-organization of informational molecules into complex molecular ecosystems that display emergent properties once thought to be unique to biological systems. The often overlooked problem of compartmentation and organismal identity will be taken up by Luisi, who will be continuing and expanding studies of self-reproducing vesicles. In a complementary approach, McCaskill has constructed open and spatially-resolved microreactors, which allow study of the continuous evolution and spatial development of self-replicating chemical systems.

The data derived from experimental studies will alternately prime and validate theoretical treatments of molecular self-replication and the processes of self-organization. Kauffman and Schuster are both pioneers in the development of theories on the self-organization of catalytic polymers. Their considerable experience will be invaluable in the development of tools for analysis of the kinetics and stabilities of the complex systems constructed by Ellington, Ghadiri, and Rebek. The interplay of experiment and theory should lead to a description of the characteristic composition of self-sustained cat-

Year 2

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<http://www.scripps.edu/chem/ghadiri/index.html>

alytic reaction networks and to the definition of chemical or informational signatures unique to each class of network. It should be emphasized that such signatures can potentially serve as the basis of identifying life elsewhere in the cosmos.

Precisely because the skills, methods, and (most importantly) the ideas required for a general description of life and its attendant signatures could never be found in a single setting, we have chosen to form a virtual Astrobiology Institute. Electronic communication and the New Generation Internet should quickly facilitate the exchange of ideas between distant researchers in seemingly disparate fields. These tools will also provide a medium for directly communicating research results to the public. A training program established under the auspices of the virtual institute will foster development of undergraduate, graduate, and postdoctoral scientists conversant in the wide array of disciplines necessary to carry the astrobiology program forward.

OVERVIEW: YEAR 2

In the past year the Scripps NAI group has made significant progress toward the stated goals of our research program. An overview of the five Scripps NAI research groups follows.

Benner's group at the University of Florida has assigned structures to organic molecules from the near-surface of Mars; provided evidence against peptide-linked nucleic acid analogs (PNAs) being a prebiotic genetic molecule; advanced efforts in *in vitro* selection with functionalized nucleic acids; assembled the Master Catalog, an evolutionarily organized database of protein sequences containing a complete history of macromolecular life on Earth; and analyzed the size of microfossils in Mars-derived meteorites.

Ghadiri's group at the Scripps Research Institute has carried out various molecular Darwinistic approaches to investigate the process of self-organization through which inanimate chemical systems may transform into the animate chemistry of living systems. This group has completed the chiroselective peptide replication studies supporting a fundamental hypothesis in the origin of biomolecular homochirality; designed and studied a new reciprocal self-replicating network; completed the synthetic phase of the first

Colin Nuckolls,
Scripps Research Institute

Liam Plamer,
Scripps Research Institute

Julius Rebek,
Scripps Research Institute

Adam Renslo,
Scripps Research Institute

Dmitry Rudkevich,
Scripps Research Institute

Alan Saghatelian,
Scripps Research Institute

K. Soltani,
Scripps Research Institute

Christopher Switzer,
University of California at Riverside

Fabio Tucci,
Scripps Research Institute

Peter Weber,
Scripps Research Institute

Yohei Yokobayashi,
Scripps Research Institute

generation molecular ecosystem; and established the essential mass spectrometric analytical tool needed for the study of the complex networks.

Ellington's group at the University of Texas Austin which carries out studies related to the evolution of self-replicating species has made contributions to the development of hybrid or chimeric replicators in which different biopolymers each contribute to a replication cycle; the development of a deoxyribozyme that is capable of self-replication from oligonucleotide substrates; and the evolution of modified ribozymes that may have augmented catalytic activities.

Rebek's group at the Scripps Research Institute aims at exploring whether or not the type of chemical instruction unique to biomolecules, such as chemical information encoded in the nucleobases of DNA and RNA leading to sequence-specific base-pairing and complex three-dimensional structure, can be designed into synthetic systems of appropriate shape, size, and functionality. Their research efforts which exploit self-complementary molecules have yielded a fluorescence energy transfer strategy to study molecular capsules based on calixarenes and new self-assembling cavitand structures. These systems are currently being optimized for studies in molecular self-replication.

Switzer's group at the University of California, Riverside seeks to synthesize alternative nucleic acids (ANAs) to attempt the optimization of polymer structure subject to the constraints of prebiotic availability, template-directed reproduction, replication conservative mutation, and fitness. Their research efforts has led to the discovery of five-stranded DNA and the finding that DNA recombination is possible with artificial Watson-Crick base-pairs. It is estimated that both of these discoveries will impact DNA's fitness with respect to information carrier and potential as a catalyst.

Overall considerable progress has been made on multiple fronts toward the central objective of our program in understanding self-reproducing molecular systems and Darwinian chemistry. Our recent findings not only significantly contribute toward the goals set forward by the NASA Astrobiology road map but are also expected to have an impact on NASA's future scientific missions to Mars and other planets. More specific summaries from each senior member of our research program is provided below.

Project

Ghadiri's Laboratory

Senior Project Investigator(s):
M. Reza Ghadiri

Ghadiri's laboratory investigates the importance of molecular Darwinistic processes in the origins of life issues by exploiting catalytic and self-reproducing (autocatalytic) polypeptide systems. The primary goal of our research program is to discover and understand factors and mechanisms that can direct the self-organization of inanimate

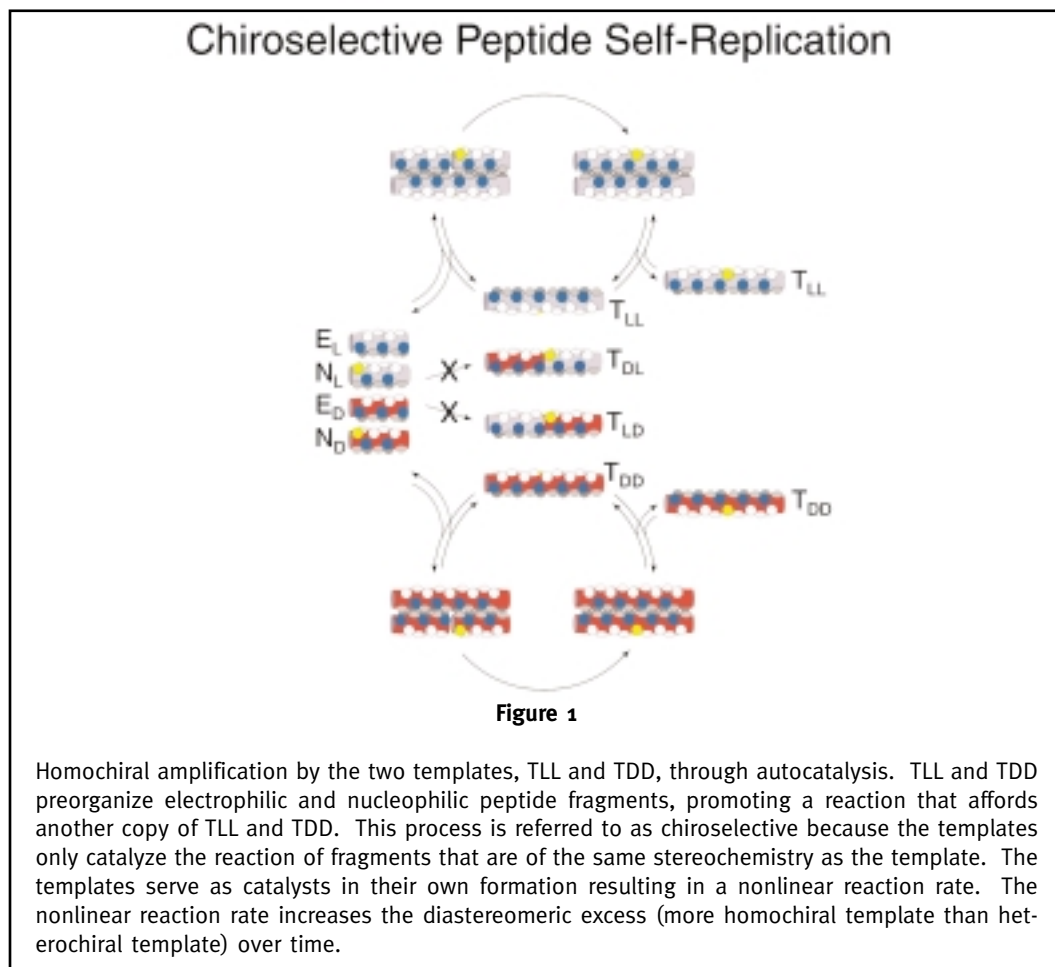
chemical transformations into the animate chemistry of living systems. Our approach has been to rationally design and recreate various forms of autocatalytic peptide networks in the laboratory and study how the interplay of molecular information and non-linear catalysis can lead to self-organization and expression of emergent properties. Our laboratory had reported previously that short helical peptides can efficiently self-replicate, in a template-dependant fashion, by catalyzing their own synthesis from appropriately functionalized shorter peptide fragments. Moreover, we had shown how such species can be employed to construct self-organized (auto)catalytic networks that can display some of the basic properties often associated with living systems such as selection, symbiosis, and error correction.

During the first year of the NAI program, Ghadiri's group: (1) developed an exponential replicator, fulfilling the structural and kinetic requirements for the onset of Darwinian evolution; (2) established the emergence of a peptide replicator in high salt, which highlights the effects of environmental factors on template-directed catalysis; (3) found the first experimental evidence for chiroselective peptide self-replication, addressing the important issue of origin of homochirality in terrestrial proteins; and (4) worked on the design, synthesis, and characterization of a reciprocal autocatalytic peptide network, which illustrates how self-reproduction can emerge out of a mutually autocatalytic set of chemical reactions.

In the past year Ghadiri's NAI team has completed the chiroselective peptide replication studies supporting a fundamental hypothesis in the origin of biomolecular homochirality; designed and studied a new reciprocal self-replicating network; completed the synthetic phase of the first generation 256-member molecular ecosystem; and established the essential MALDI-TOF mass spectrometric analytical tool needed for the study of the complex networks by designing a new matrix system for the simultaneous and quantitative analysis of multiple peptide species in complex reaction mixtures. These advances are briefly described below.

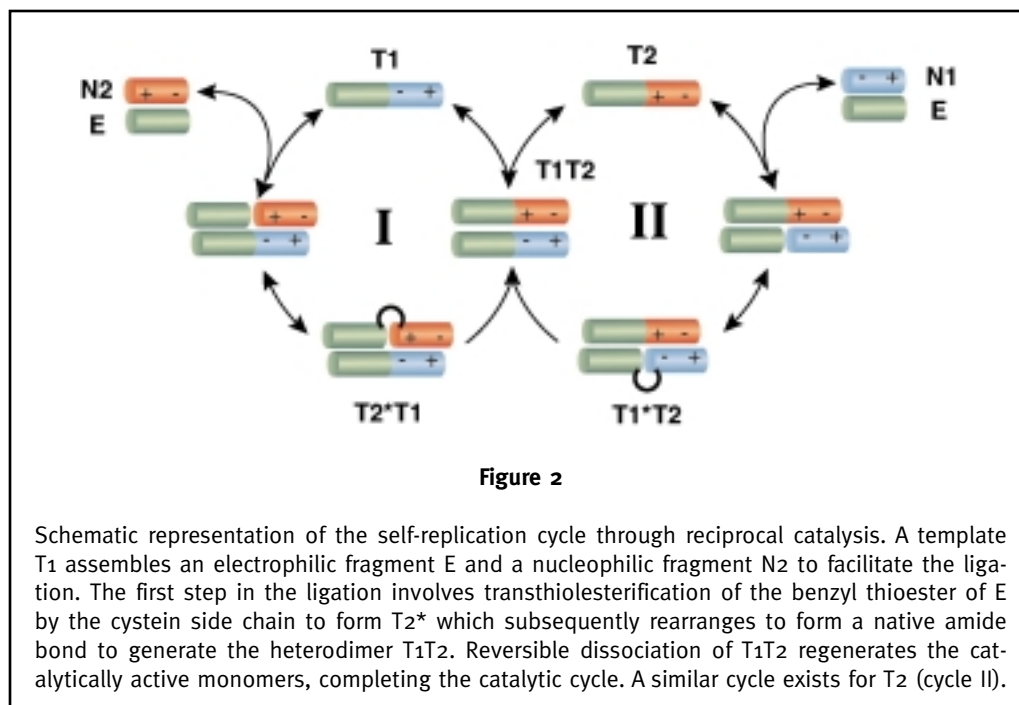
1. Chiroselective peptide self-replication. It has been argued that homochirality is a necessary condition for self-reproducing prebiotic molecular systems. However, despite its central importance in the origins of life theories, the feasibility of chiroselective amplification in biopolymers had not been established. Previous attempts aimed at demonstrating template-directed homochiral replication of nucleic acids and its analogues have proven inconclusive due to product or cross-isomer inhibition processes. We have demonstrated that a peptide replicator can amplify efficiently and specifically homochiral products through a chiroselective autocatalytic cycle. Moreover, the system is shown to be remarkably robust with even a single stereochemical mutation inhibiting the self-replication process supporting the postulate that homochirality is a necessary condition for self-replication.

2. Reciprocal self-reproducing networks. Studies on the non-enzymatic self-replication of synthetic molecules have provided a great deal of insight into the theoretical and experimental approaches to the origin of life. However, a living system is a far more complex network of chemical reactions. In attempts to further our understanding of how the complexity and emergence in living systems arise, various models of chemical networks based on self-replicating molecules have been constructed. The two most recent



designs that we have completed comprise of two peptides neither capable of self-replication. However, when mixed together, the system as a whole reproduces due to the reciprocal catalysis afforded by its products. These peptides constitute the first implementations of the minimum three-component reciprocal network. It remains to be seen how such reciprocal networks behave in the context of more complex systems.

3. Design of a 256 member molecular ecosystem. One of the major goals of our research program has been to construct a molecular ecosystem and study the behavior of a large collection of catalytic and autocatalytic peptides, the process of self-organization, and network formation under variety of starting conditions and environmental stimuli. We have completed the synthetic phase of the first generation ecosystem. The system is composed of 16 electrophilic and 16 nucleophilic peptide fragments that when combined would yield 256 distinct mass-encoded peptide condensation products. Our immediate objective in employing this relatively large molecular population is the following: a) to investigate the production of efficient replicators, and b) to determine in how many ways and under what selection pressures complex self-organized systems may arise. Moreover, we will explore whether or not the first generation peptide ecosystem can reorganize (adapt) to changes in its environmental conditions such as pH, ionic



strength, temperature, etc. The main practical obstacle in the study of a dynamic molecular ecosystem is how to find the peptides and networks of interest amongst the many non-fertile sequences and unproductive background processes that are expected to occur in such complex reaction mixtures. Current high performance chromatographic analysis techniques would not be useful in such complex cases since most of the peptides employed possess similar physical characteristics. Our strategy has been to use the rapidly advancing technology of MALDI mass spectrometry to follow the behavior of every peptide within the population and discover the peptides and network organizations of interest. By mass spectrometry one should be able to quantitatively monitor the production of each peptide if each member of the population is represented by a unique molecular mass. As described briefly below we have recently developed the required methodology to exploit mass spectrometry for the analysis of the molecular ecosystem.

4. Quantitative analysis of multicomponent catalytic and autocatalytic peptide fragment condensation reactions by MALDI-TOF mass spectrometry. The quantitative study of these multicomponent reaction systems is a challenging endeavor since most of the existing methods are either very time-consuming or limited to the analysis of a small number of species in solution. For example, reversed-phase high-performance liquid chromatography (RP-HPLC) as the method of choice for the analysis of autocatalytic networks can only be used for systems composed of approximately four starting components yielding about the same number of products. This is due to the limited resolution of HPLC and the chromatographic "similarity" of typical analytes such as DNA or peptides. As a prerequisite for a research program directed at the design, analysis, and discovery of novel self-organizing chemical networks of higher complexity, we have sought to evaluate the ability of modern matrix-assisted laser desorption/ionization time-of-

flight mass spectrometry (MALDI-TOF-MS) for multicomponent reaction profile analyses. Multicomponent peptide self-replication reactions based on the templating effect of coiled-coil peptides were monitored quantitatively with MALDI-TOF-MS. A large number of matrices in combination with a variety of co-matrices were screened against a model mixture of four different peptides to obtain similar ionization intensities, despite significant differences in their amino acid composition and aggregation tendencies. The novel combination of 2-mercaptobenzothiazole with tris(2-carboxyethyl)phosphine as the MALDI matrix system was found to be the most efficacious in all mixtures studied. Under these conditions strong signals are observed even in the presence of large amounts of sample buffer and other contaminants. The utility of the system was demonstrated by the detailed study of several peptide replication reactions. Up to 16 individual product peptides that were formed simultaneously in a reaction mixture could be quantified accurately. The results raise the prospect of further applications of MALDI-MS for the studies of multicomponent reaction systems of even higher complexity such as the one planned for the study of molecular ecosystem as well as fast quantitative analyses in the field of proteomics and combinatorial chemistry.

Project

Benner's Laboratory

Senior Project Investigator(s):
Steve Benner

In the past year, the Florida Astrobiology Group has made five major contributions towards the goals set forward by the NASA Astrobiology Roadmap. We have: (1) assigned structures to organic molecules from the near-surface of Mars; (2) provided evidence against peptide-linked nucleic acid analogs (PNAs) as being a prebiotic genetic molecule; (3) advanced efforts to do *in vitro* selection with functionalized nucleic acids; (4) assembled the Master Catalog, an evolutionarily-organized database of protein sequences containing a complete history of macromolecular life on Earth (as it can be presently inferred from genomic sequence data); and (5) analyzed the size of microfossils in Mars-derived meteorites.

1. Organic Molecules on Mars. The NASA Planetary Exploration Missions. Entirely funded by the NAI, the Florida Astrobiology sub-node has combined theoretical and experimental work to suggest that, contrary to the widely held interpretation of experiments from the Viking 1976 landers, organic molecules should be present on the surface of Mars. This work is influencing the design of Mars probes. After extensive discussions with scientists involved in the 1976 Viking mission, a paper describing this result appeared in *Proc. Natl. Acad. Sci. USA*. Within the month, Luann Becker (Univ. Hawaii) delivered to us her laser desorption mass spectra from Allan Hills and Nakhla meteorites, both derived from Mars. These spectra show the presence of organic matter in these meteorites. C_{12} - C_{13} isotope ratio measurements suggest that this organic material was delivered to the rocks while they were on Mars, from kerogen contained in meteorites that landed on Mars.

Interestingly, both meteorites generate a mass spectrum with a peak at 288. This is the mass of the trianhydride of benzenhexacarboxylic acid, which is expected to be the principal compound observed by laser desorption mass spectrometry in any sample containing benzenhexacarboxylic acid. Benzenhexacarboxylic acid is the principal organic compound that we predicted should be present on the surface of Mars.

While one is obligated to distrust any experimental result that one wants to believe, if the 288 peak turns out to arise from benzenhexacarboxylic acid, this will be a very short turn-around between prediction and verification. It also serves as a nice illustration of how a broad understanding of organic chemical reactivity can be useful to NASA in general and to the NAI in particular.

More importantly, however, close inspection of the mass spectra of the high molecular weight material from ALH and Nakhla reveals significant differences. Naively, these differences suggest that the organics in ALH were exposed to molecular oxygen over very long periods of time, while those from Nakhla were not. ALH is a much older rock, formed over 4 billion years ago. Nakhla is much younger, perhaps less than 2 billion years old. While speculative, it is intriguing to note the possibility that the differences in the high mass organic products may indicate the presence of oxygen in the atmosphere of Mars in a time when oxygen was appearing first on Earth as a result of biogenesis.

At this early stage, we certainly do not suggest that this can be interpreted as evidence for life on Mars 3 billion years ago. These experiments have, however, opened up a line of reasoning that might conclude this, especially if more SNC meteorites are investigated, and if samples ultimately become available from Mars. In the next year (see below) we plan to start to build the organic chemical tools that will enable such a conclusion to be drawn.

2. Universal Chemical Features of Genetic Biopolymers. Consistent with our focus as stated in the last report, and partly funded from Astrobiology sources, the Florida Astrobiology sub-node has developed further its "second generation" model of nucleic acids that emphasizes the importance of the polyanionic nature of DNA as a genetic biopolymer. The concept of a biopolymer that is Capable of Suffering Mutation Independent of Concern over Loss Of Properties Essential for Replication (COSMIC-LOPER) has been adopted by other authors in their effort to understand self-organization and self-replication in molecular systems. COSMIC-LOPERness permits a biopolymer to support a Darwinian search for function. A polyelectrolyte structure (polyanionic or polycationic) is proposed to be a universal feature of the genetic molecules of life, regardless of genesis. This work all but excludes peptide nucleic acid analogs (PNAs) as the original genetic material. A probe to search for such structures in planetary missions is being designed.

3. Darwinian Chemistry. Also consistent with our focus as stated in the last report, we further developed functionalized standard and non-standard DNA molecules as the starting point for generating biological function. The goal continues to be to develop molecular systems that can direct the synthesis of replicates with the possibility of mutation, selection, and therefore evolution, and to quantitatively analyze their performance in *in vitro* selection experiments. Largely funded from non-Astrobiology sources, but central

to the Astrobiology program's goal of obtaining self-replicating systems in the laboratory, the work from the Florida Astrobiology sub-node that examined *in vitro* selection "structure space" in both functionalized and non-functionalized nucleic acids. These studies represent a substantial step in our progress towards self-replicating systems in the laboratory.

4. The Master Catalog, a Comprehensive Model for Life in the Terrestrial Biosphere. Largely funded from non-Astrobiology sources, but central to the Astrobiology goal of describing the history of life on Earth, the Florida "Master Catalog" has identified all of the independently evolving modules from completed microbial genomes, and built for each an evolutionary history, an analysis of the evolution of function, and a preliminary secondary structural model. The dataset is now being analyzed to better understand the microscopic processes by which proteins evolve. At present, the Master Catalog is accessible free of charge to interested scientists, and soon it will be offered as a commercial product by the company EraGen Biosciences. This tool underlies our strategy of "working backwards in time", a stepwise approach that proceeds from more highly validatable models of the history of Earth to more controversial ones farther back in time.

5. Structures in the Allan Hills Martian Meteorite. Not too Small to be Living Cells. Entirely funded by the Astrobiology program, the Florida Astrobiology sub-node has joined theory and experiment to show that the structures in the Allan Hills meteorite are not too small to be remnants of life, if that life is based on a single biopolymer capable of both genetics and catalysis. Such biopolymers are believed to have enabled the origin of life on Earth. A 50-step metabolism for small cells has been proposed from an understanding of organic reactivity. The paper has now appeared.

Project

Ellington's Laboratory

Senior Project Investigator(s):
Andrew Ellington

The Ellington lab is carrying out work in three areas related to the evolution of self-replicating species: the development of hybrid or chimeric replicators in which different biopolymers each contribute to a replication cycle, the development of a deoxyribozyme that is capable of self-replication from oligonucleotide substrates, and the evolution of modified ribozymes that may have augmented catalytic activities.

1. RNA Ligations on Peptide Templates. Self replicating systems based on nucleic acids as well as α -helical peptides have previously been demonstrated. In order to further understand self-replicating molecular ensembles we have been investigating a hybrid system in which we are attempting to generate an interdependent set of self-replicating nucleic acids and peptides. To do this we have been using specific RNA sequences (aptamers) that have been evolved *in vitro* to bind specific peptide targets. As a start-

ing point in our replication cycle we have been investigating the ability of these peptides to serve as templates for the ligation of an aptamer that has been broken into two fragments.

We have found using the activating agent cyanogen bromide that ligation of two RNA aptamer fragments is specifically enhanced up to ~10 fold in the presence of the aptamers cognate peptide. While nonspecific reaction between peptide and RNA can also occur this can be suppressed by the addition of a non-specific competitor RNA such as tRNA. Our overall yield for the ligation reactions however are low (~1-5%), and so we are currently investigating alternative ligation schemes such as using preformed phosphorimidazolides and N-hydroxybenzoic esters.

2. DNA Ligase That Forms An Unnatural Internucleotide Linkage. We have been investigating the ability of DNA to catalyze a ligation reaction between an oligonucleotide bearing a 5' iodine and another containing a 3' phosphorothioate. The resulting 5'phosphorothioether linkage is analogous to the normal phosphodiester linkage and behaves similarly both chemically and structurally.

The general ligation selection scheme is based on the selection scheme used by Shoztak and Bartel. The pool contains a random region of 90 nucleotides. After 13 rounds of selection the rate of the ligation reaction was enhanced ~500 fold.

Sequencing after round 10 showed two dominant clones which each appeared 3 times out of 20 sequences (class Ia and class II). An additional round was conducted to see if these two clones would take over the population. Round 11 yielded one dominant clone from class II, and no members of class Ia. Surprisingly this clone was the slower of the two clones identified in the previous round. This may be a result of a preference of certain clones for specific substrates (see below). After two additional rounds of selection, the pool was again sequenced. This time the dominant clones were from class Ia, the faster of the two dominant clone families from round 10. Although five different substrates were used during the course of the selection, individual clones show a substrate preference. The switch between dominant clones in the latter rounds of the selection may be related to their relative substrate preferences.

In order to begin to understand what regions of the selected DNA enzyme were necessary for the ligation reaction, a ladder of 3' deletion mutants was constructed for both class Ia and class II DNA ligases. The deletion mutants were then assayed for their ability to catalyze the ligation reaction. The 3' ends of the cannot be deleted much past the position of the 3' primer binding site indicating that the 3' end of the molecule is important for catalysis. It is not surprising that the primer binding region itself is nonessential as it is double stranded during the selection and therefore less available for bonding interactions.

We are currently trying to address other structural characteristics of the class Ia and class II DNA ligases by performing a doped selection. This will allow for identification of regions of co-variation and a better understanding of the structural details of these classes of ligases.

The relatively low rate enhancement for the ligation reaction may be a result of the inability of DNA to stabilize the iodine leaving group. This may also be a factor for RNA

enzymes. Both the uncatalyzed and catalyzed ligation reactions appear to be Mg^{2+} independent. The catalyzed reaction shows only a ~2 fold difference in its absence. The inability to use this metal may be the result of the poor affinity the hard Mg^{2+} for sulfur containing compounds. Replacing Mg^{2+} with the more thiophilic Mn^{2+} has little effect on the reaction rate. Other metals which may affect catalysis are Ag and Hg, which can be used specifically to cleave the 5'phosphorothioether linkage. We are currently planning another selection using this same pool and a variety of metal ions in hope of identifying faster ligators. A comparison of metal dependent and metal independent ligators will allow us to begin to understand some of the limitations of nucleic acid catalysis as well as address the role of metal ions in enhancing the chemical catalysis necessary to support early metabolism and the first replicating systems.

In the next few months we hope to further characterize the structure and catalytic function of the class Ia and class II ligators. Essential to this is a doped sequence selection which is now in progress. Our new knowledge of structural information will be used to redesign the ligators so that they are capable of catalyzing a ligation reaction in trans. We will then begin to look for ways to break the ligators so that they become substrates for this trans ligation reaction. In this way we hope to be able to design a novel self-replicating ligase enzyme in which two or more nonfunctional fragments are joined to form an active trans ligator capable of performing additional ligation reactions.

3. Evolution of Ribozymes with Modified Nucleotides. We have synthesized modified nucleoside triphosphates, including 5-hydroxymethyl uridine triphosphate, 5-amidizolemethyl uridine triphosphate, and 5-phenolmethyl uridine triphosphate. We now have available 10-20 micromole amounts of these nucleoside triphosphates that can be used for initial experiments. Each of these modified nucleotides can be readily incorporated into transcripts and pools by T7 RNA polymerase. We have recently synthesized multi milligram amounts of the MN90 pool in which each of these nucleotides has been substituted for uridine to carry out selection experiments. The modified transcription products have slightly altered mobilities by gel electrophoresis, as expected. We will now carry out RNase T2 digestions and isolate products by HPLC to confirm the incorporation of the modified nucleotides and to estimate the level of contamination by uridine, if any. We will then begin selection experiments for ligators. These experiments will be carried out in parallel with a pool containing unmodified ribotides in order to directly compare the catalytic augmentation of RNA by modified nucleotides.

Rebek's Laboratory

Project

Senior Project Investigator(s):
Julius Rebek Jr.

The nucleobases A, C, G, and T encode the instructions for the sequence-specific base-pairing that ultimately results in the double-helical structure of DNA. Are complex structure/assembly relationships predictable and unique to biomolecules, or can they be exploited in synthetic systems of appropriate shape, size, and functionality? As our own systems become more complex, can we study, understand, and even anticipate their behavior or function? These are a few of the questions that fuel our current research efforts. The two projects highlighted here feature suitably-functionalized, self-complementary molecules that form assemblies held together by weak, non-covalent interactions. In the first case, a fluorescence energy transfer strategy is employed to study molecular capsules based on calixarenes. Not only does the technique provide mechanistic and behavioral insight into the assembly (dimerization) process, but it also provides a basis for the sensitive detection of small molecules in solution. The second example describes further work on a recently introduced "ball-in-socket" assembly of cavitands.

The routine spectroscopic techniques often used to characterize synthetic assemblies (e.g., NMR) become inadequate when the systems get large and are composed of multiple species. We have recently devised a fluorescence protocol to investigate the association and dissociation processes of our calixarene capsules (Figure 1a). When assembly occurs between fluorophore-tagged monomers, fluorescence resonance energy transfer (FRET) occurs upon their assembly. The process can be monitored in real time at two wavelengths (Figure 1b), and both the monomers and assembly can be observed directly. From our studies we have learned that molecules of this type behave much differently at nanomolar concentrations than they do at the millimolar concentrations used in NMR analysis. We have recently published these findings.

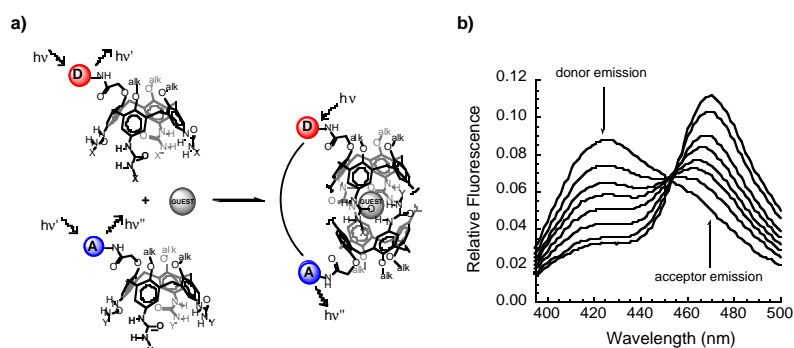


Figure 1

Further studies have shown that the technique is useful in the sensitive detection of small molecules. The assembly process of Figure 1a only occurs in the presence of a suitable guest molecule (which is often just the solvent). If the solvent is a poor guest, then assembly does not take place; addition of a better guest nucleates the dimer and a fluorescence signal results. Representative results are shown in Figure 2. When the two fluorophore-tagged monomers are dissolved in p-xylene no assembly occurs, and there is no emission signal at 450 nm. Addition of 3-methylcyclopentanone, an unlabeled analyte, results in energy transfer with added equivalents of the guest (equiv.). Future assemblies could conceivably be targeted toward specific molecules for detection.

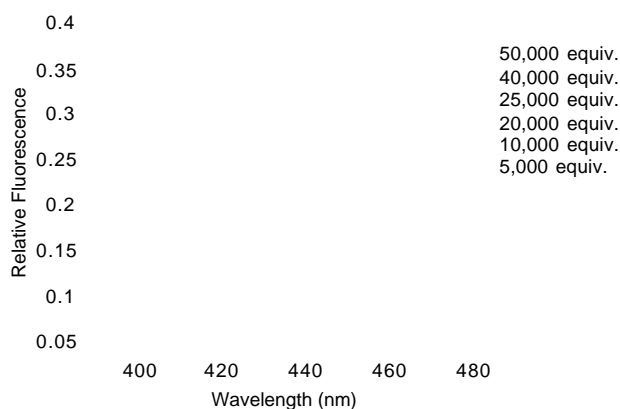


Figure 2

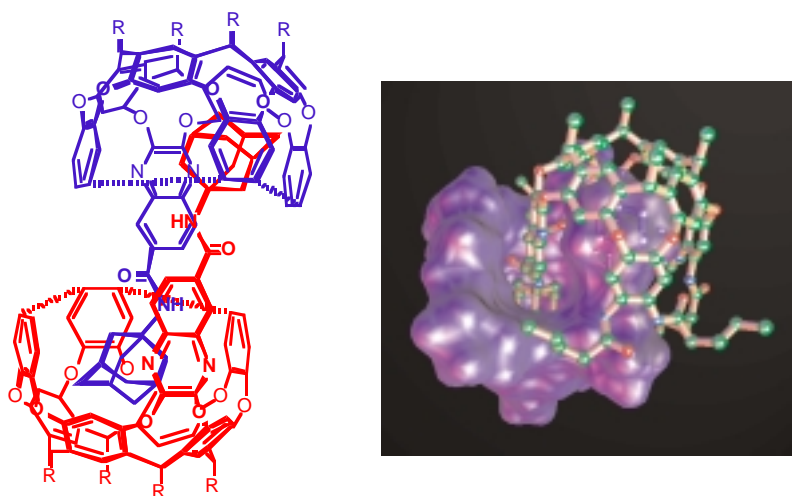
**Figure 3**

Figure 3 shows an assembly that is constructed from cavitands with "ball-in-socket" complementarity. Here the adamantane "guest" of one molecule fits inside the bowl-shaped portion of another identical molecule, and a stable dimer results. Recent work has shown that there is a huge entropic cost to forming the assembly, but the price is paid by favorable guest-host contacts. The dimer is unusual in that it is driven to formation by van der Waals contacts and the filling of space. Our current efforts are directed toward self-replicating systems based on this motif.

Switzer's Laboratory

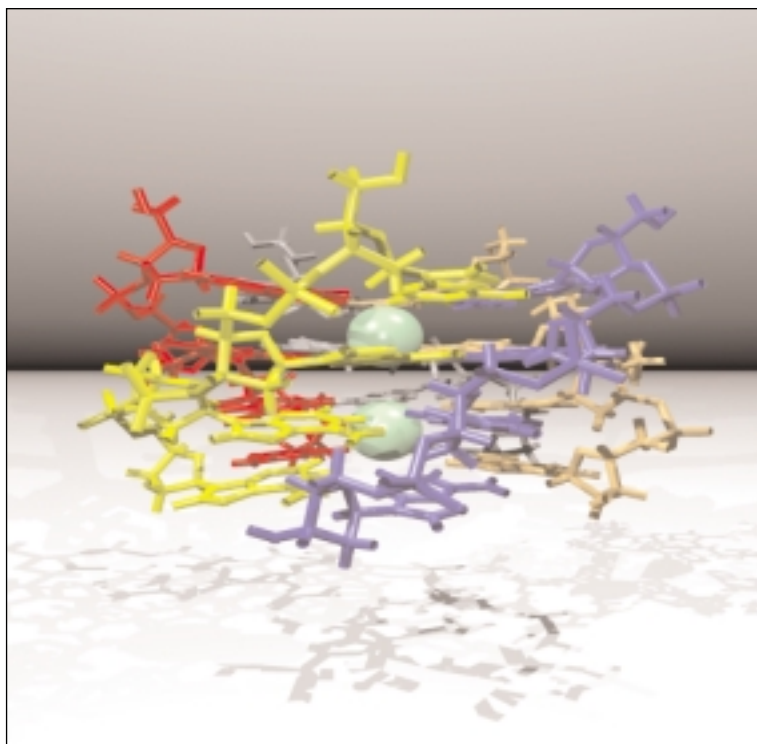
Project

Senior Project Investigator(s):
Christopher Switzer

Our primary goal is to synthesize Alternative Nucleic Acids (ANAs) to attempt the optimization of polymer structure subject to the constraints of prebiotic availability, template-directed reproduction, replication conservative mutation, and fitness. We have identified ANAs by taking small steps in "structure-space" away from RNA (the best model for a molecule bearing features both universal and unique to life) that may avoid some of the problems inherent in fulfillment of the aforementioned constraints. Specifically, the Switzer laboratory is examining ANAs with novel perturbations to (i) base-pairing domains and (ii) formal charges. These studies will help to define chemi-

cal parameters for molecular evolution. Furthermore, our work addresses whether nucleic acid-like molecules are sufficient to enable the origin of life and what limitations exist for life elsewhere in the universe based on a single biopolymer (e.g. RNA) rather than multiple biopolymers (DNA, RNA, proteins, carbohydrates). Accomplishments by our group for the past year are highlighted below.

1. Discovery of a DNA Pentaplex (Chaput, J. C., Switzer, C. "A DNA Pentaplex Incorporating Nucleobase Quintets," *Proc. Natl. Acad. Sci., U.S.A.*, (1999) 96, 10,614-10,619). In the past year we have discovered a new structural form of DNA -- a pentaplex. In particular, our group has found that DNA bearing the purine component of a non-standard Watson-Crick base-pair (the iso-G:iso-C base-pair, used in past work to expand the genetic code) can form five-stranded helices via nucleobase quintets in the presence of monovalent cations, such as cesium ions. Not only does the DNA pentaplex go beyond the previous four-strand limit for a DNA helical assembly, it also raises the possibility further expanding DNA helical structures to hexaplexes or beyond. By way of background, peptides, in contrast to nucleic acids, routinely give structures with high degrees of strand association. The greater tendency seen for peptide strands to aggregate as compared to nucleic acids is on the one hand consistent with the gross physical properties of the two biopolymers -- nucleic acids bear high negative charge densities for which repulsive coulombic forces must be balanced, and this is more difficult to do as more strands are combined. Peptides generally have diminished charge densities relative to nucleic acids. However, our work demonstrates DNA's previous inability to combine beyond a tetraplex was not due to a general molecular property (e.g., the repeating negative charge) but rather a result of a particular molecular feature (the positioning of functional groups on nucleobases). Our work further demonstrates that features common to DNA found in terrestrial genomes impose artificial limits on DNA structural behavior -- limits that may not pertain to nucleic acid-like polymers either on the prebiotic Earth or extraterrestrially. The increase in nucleic acid structural diversity conferred by the pentaplex (and possibly even higher order structures) is sure to impact DNA's fitness landscape with respect to both information carrier and potential as a catalyst. A goal of our laboratory is to gauge this impact.



Five-Stranded DNA Complex

2. Recombination with an Expanded Genetic Alphabet (Rice, K., Chaput, J. C., Cox, M. M., Switzer, C. "RecA Protein Promotes Strand Exchange with DNA Substrates Containing Isoguanine and 5-Methyl Isocytosine" *Biochemistry*, (2000), in press). Recombination mechanisms have the potential of imposing serious limitations on the fitness of nucleobases beyond those presently found in terrestrial genomes. This is true since selective advantages for organisms that utilize recombination competent genetic materials include an additional means for DNA repair, and the capacity for sexual, as opposed to asexual, reproduction. The *Escherichia coli* RecA protein pairs homologous DNA molecules and promotes DNA strand exchange and recombination *in vitro*. As a model for recombination, we have examined DNA strand exchange between a 70 nucleotide single-stranded DNA fragment and a 40 base-pair double-stranded DNA fragment, in which all G and C residues (at 18 positions distributed throughout the 40 bp exchanged region) were replaced with the non-standard, isomeric nucleotides iso-G and iso-C. It is found that the nonstandard oligonucleotides are substrates for the RecA protein, permitting DNA strand exchange *in vitro* at a rate and efficiency comparable to exchange with normal DNA substrates. This observation provides an expanded experimental basis for discussions of potential roles for iG and iC in a genetic code.

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UNIVERSITY OF CALIFORNIA, LOS ANGELES

Los Angeles, California



PRINCIPAL INVESTIGATOR



Bruce Runnegar

UCL TEAM MEMBERS

Eric Becklin,

University Of California Los Angeles

Andrew Czaja,

University Of California Los Angeles

Katherine Ellison,

University Of California Los Angeles

James Farquhar,

University of California San Diego

James Ferry,

Pennsylvania State University

Sorel Fitz-Gibbon,

University Of California Los Angeles

Ruth Gates,

University Of California Los Angeles

James Gehling,

University of South Australia

Andrea Ghez,

University Of California Los Angeles

Michael Ghil,

University Of California Los Angeles

Dara Goldberg,

University Of California Los Angeles

PROPOSAL SUMMARY

The origin of life, its occurrence in the Universe, and the nature of early life on Earth are outstanding frontiers of human knowledge. UCLA is exceptionally well placed to address these issues because it fosters a group of talented scientists with complementary skills in evolutionary biology, microbiology, molecular biology, biochemistry, isotope geochemistry, geology, astronomy, planetary science, space physics, atmospheric sciences, earth system science, and nonlinear dynamics. All of these scientists work and teach cooperatively and collaboratively. Institutional strengths include: (1) the IGPP (Institute of Geophysics and Planetary Physics) Center for the Study of Evolution and the Origin of Life (CSEOL); (2) the UCLA ACCESS program for interdepartmental graduate education in molecular biology; (3) the W.M. Keck Foundation National Center for Isotope Geochemistry; and (4) major partnerships in the Keck Foundation and SOFIA (Stratospheric Observatory for Infrared Astronomy) observatories. Thus UCLA, in concert with other sectors of the University of California, is in a unique position to make a powerful contribution to the quest for knowledge of the origin and nature of life both in deep space and in deep time.

In order to pursue these goals, UCLA is committed to developing astrobiology as a discipline for the 21st Century. In addition to providing nearly three quarters of a million dollars in matching funds, there will be a new faculty position in astrobiology, new programs to recruit and train graduate students, new opportunities for postdoctoral education, and a new focus in general education. UCLA aims to create an environment that will stimulate and sustain a cross-campus adventure in research and education. We see the formation of the NASA Astrobiology Institute as an unprecedented opportunity for intellectual growth and collaborative research within UCLA, across the UC System, and in partnership with other members of the Astrobiology Institute.

UCLA scientists and students will focus on three principal sources of information about the origin and evolution of life in the Universe. These are the fossil record, microbial evolution, and the search for life outside the Earth. In each of these areas, UCLA scientists have considerable experience from research work in: (1) Earth's earliest biospheres and the environmental context in which they evolved; (2) the earliest history of

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living microorganisms using gene sequences and whole genomes; (3) designing space-craft instruments for *in situ* exobiological exploration; and (4) developing instrumentation and techniques for advanced telescopes. Immediate goals of proposed research are: (1) a search for habitable planets outside our solar system using a combination of theory, observation, and experiment; (2) an integrated examination of Earth's earliest life using the combined resources of ion microprobe biogeochemistry, genomics, and micropaleontology; and (3) the training of young people for the decades ahead when opportunities for explorative searching for extraterrestrial life will become commonplace. For the long term, UCLA wishes to be an active and productive member of the NASA Astrobiology Institute.

PROPOSAL EXECUTIVE SUMMARY

UCLA is poised to lead the University of California's research and educational program in astrobiology (UC Center for Astrobiology), building on existing strengths and extensive experience in paleobiology, exobiology, astronomy, and planetary science. Sixteen members of the permanent faculty, including the Directors of the Center for Study of Evolution and the Origin of Life (CSEOL), the Institute of Geophysics and Planetary Physics (IGPP), the W.M. Keck Foundation National Center for Isotope Geochemistry, and the Stratospheric Observatory for Infrared Astronomy (SOFIA), together with senior and junior professors from the Departments of Biology, Earth and Space Sciences, Physics and Astronomy, and the Molecular Biology Institute are enthusiastically committed to developing astrobiology -- the discipline -- within the University of California System. This group believes that astrobiology is the science and technology for the 21st century, which they propose to advance through innovative, cooperative, and cross-disciplinary research and education, all in the splendid tradition of great American research universities.

The institutional commitment to this endeavor is equally substantial. In addition to providing research space and 0.7 million dollars in matching funds for the first five years of the program, there will be a new faculty position in astrobiology, new programs to recruit and train graduate students, new opportunities for postdoctoral education, and a greater emphasis on existing undergraduate courses in astrobiology. As UCLA is cur-

Kathleen Grey,
Geological Survey Of Western Australia

T. Mark Harrison,
University Of California Los Angeles

Andrew Hock,
University Of California Los Angeles

Seth Hornstein,
University Of California Los Angeles

Christopher House,
Pennsylvania State University

David Jacobs,
University Of California Los Angeles

Ravi Jain,
University Of California Los Angeles

Per Jögi,
University Of California Los Angeles

Patricia Johnson,
University Of California Los Angeles

Michael Jura,
University Of California Los Angeles

Keith Kirts,
University Of California Los Angeles

Frank Kyte,
University Of California Los Angeles

David LaFreniere,

University Of California Los Angeles

James Lake,

University Of California Los Angeles

Crispin Little,

University Of California Los Angeles

Donald Lowe,

Stanford University

James Lyons,

University Of California Los Angeles

Craig Manning,

University Of California Los Angeles

Caer-Eve McCabe,

University Of California Los Angeles

Chris McCarthy,

University Of California Los Angeles

Kevin McKeegan,

University Of California Los Angeles

Ian McLean,

University Of California Los Angeles

Jeffrey Miller,

University Of California Los Angeles

Michael Mischna,

Pennsylvania State University

Steve Mojzsis,

University Of California Los Angeles

Jonathan Moore,

University Of California Los Angeles

William Moore,

University Of California Los Angeles

William Newman,

University Of California Los Angeles

David Paige,

University Of California Los Angeles

Maria Rivera,

University Of California Riverside

rently rethinking and reorganizing its whole approach to undergraduate general education in the context of national concern over the assimilation of technical and cultural knowledge by young Americans, the multidisciplinary nature of astrobiology coupled with its broad appeal make it a prime candidate for cross-campus communication. As all UCLA students have direct 24-hour access to the internet through BruinOnLine, and all courses at UCLA have mandatory web page support, it will be immediately feasible to bring the information and experiences of the NAI high-speed network (NGI: New Generation Internet), and NAI itself, into the classrooms, laboratories, and dormitories.

With regard to the proposed research program in astrobiology, UCLA scientists will focus on the three principal sources of information about the origin and evolution of life in the Universe. These are the fossil record (on Earth or elsewhere), comparative microbiology (from morphometrics to genomics), and the remote sensing of habitable environments and life itself. In each of these areas, UCLA has considerable experience through: (1) work on Earth's earliest biospheres and the environmental context in which they evolved; (2) research into the deepest history of living microorganisms using gene sequences and whole genomes; (3) designing and deploying instruments for spacecraft, including the upcoming UCLA/JPL/University of Arizona MVACS (Mars Volatiles and Climate Surveyor) mission to Mars; and (4) developing the instrumentation and techniques for advanced telescopes at the SOFIA and Keck observatories.

As the Astrobiology Institute matures in the years to come, UCLA scientists will search the galaxy and explore the solar system for evidence of the existence of extraterrestrial life. In the meantime, Earth's own ancient past may be used as a natural laboratory to find out how life emerged and how it alters the environments in which it evolves. The knowledge gained will not only provide insights into the nature and antiquity of life on Earth, but it will also suggest better ways to detect and characterize life on other worlds.

Until now, microfossils and the chemical signature of carbon fixation have provided independent clues to the nature of Earth's earliest life. These approaches may now be combined through use of the Keck Center's high resolution ion microprobe, which has unique capabilities for determining the *in situ* isotopic compositions of common biosynthetic elements (C, N, O, S) from microfossils and their degraded remnants. On one hand, this opens the possibility of sampling extraordinarily small volumes of refractory ancient crust (e.g., inclusions in zircon crystals) that have been incorporated into the oldest geological terranes for paleoenvironmental data. Such research sampling would extend the rock record from the earliest Archean (~4.0 billion years ago) into the previously unknowable Hadean Eon. The alternative is to measure the isotopic compositions of individual microfossils that can be related by their morphological and chemical properties to the living biota. A comprehensive survey of the morphologies and morphometrics of recently discovered living micro-organisms -- especially those that inhabit extreme environments and produce distinctive isotopic fractionations by known metabolic pathways -- will be used to interpret the ion microprobe results from the Precambrian rock record. This research involves close collaboration among isotope geochemists, geologists, microbiologists, and paleobiologists. It has obvious applications to the search for evidence of life on Mars and other rocky bodies.

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Although the topology of the tree of life on Earth is now well known (from comparisons of small subunit ribosomal RNA genes from a comprehensive sample of the biosphere), the dating of crucial branch points (nodes) in terms of the geological time scale is still in its infancy. Furthermore, it is not clear how much of the earliest history of life is summarized in the attributes of living organisms. Paleontologists, for example, argue that evolution proceeded extraordinarily slowly during the three billion years of microbial history that predated the appearance of complex multicellular life. They also argue that the last common ancestor of all living organisms may be older than the preserved rock record. Alternatively, some molecular biologists think that the last common ancestor lived comparatively recently (~2 billion years ago). In that case, older fossils may sample an extinct biosphere that is poorly represented in the modern world.

Twelve complete genomes of a wide array of living micro-organisms including yeast, three archaeobacteria, and eight bacteria are now sequenced. Many more will appear during the next few years. Given the compactness of individual genes (and in many cases, their conservation over much of the history of life), these genome sequences represent vast stores of phylogenetic information. (Sequences so far available total about 40 megabases.) Analysis of these data will be aimed at reconstructing the genome of the last common ancestor of all living things and those of key nodal organisms in the tree of life. It is already clear that there are two functionally distinct classes of genes, a deeply diverging informational lineage that is largely immune to lateral transfer and an operational suite that records gene transfer events. For vital genes, these lateral transfers are rare enough to serve as time lines across the tree of life. Lateral gene transfers (in combination with new molecular clock methods, adding confidence intervals to fossil ranges, and obtaining precise U-Pb ages) will be used to correlate and calibrate key nodes in the tree of life. The challenge is to go beyond the last common ancestor by integrating genomic and geochemical evidence for Earth's earliest life.

UCLA astronomers and space scientists have access to extraordinary facilities for remote and *in situ* exploration for extraterrestrial life. The Keck and SOFIA observatories will be used to study the carbonaceous dust clouds (protoplanetary disks) around main sequence and pre-main sequence stars. Nearby (50 parsecs) and very young (20 Ma) T Tauri stars are a natural laboratory for planet formation, including the initial stages of rocky body assembly. At the other end of stellar evolution, heavy elements in the spectra of white dwarfs may provide evidence for the existence of comet clouds. Oort-like comets are produced by a particular class of planets that are otherwise undetectable. These and other evidence for stellar systems will be used to develop theoretical models for planetesimal accretion in the presence of giant planets in inboard orbits. The simulations will model swarms of planetesimals, adhesion following collisions, and the long-term dynamical stability of terrestrial protoplanets. Laboratory experiments aimed at understanding radioactive heating of planetesimals will be used to constrain the timing of the numerical simulations. This synergistic approach (involving experiment, modeling, and observation) is expected to enhance the search for likely abodes for life.

UCLA is responsible for the MVACS (Mars Volatiles and Climate Surveyor) integrated payload for the Mars Surveyor Program 98 Lander mission to Mars (1999-2000). This payload consists of four instruments that will provide information about the current surface environment and history of the volatile inventory of Mars from a south polar landing

Andrew Roger,
Dalhousie University

Bruce Runnegar,
University Of California Los Angeles

Matthew Saltzman,
Ohio State University

J. William Schopf,
University Of California Los Angeles

Gerald Schubert,
University Of California Los Angeles

Jeffrey Silberman,
University Of California Los Angeles

Insoek Song,
University Of California Los Angeles

Karl Stetter,
Universität Regensburg

Ferenc Varadi,
University Of California Los Angeles

Michael Vondrasco,
University Of California Los Angeles

M. Indira Venkatesan,
University Of California Los Angeles

Malcolm Walter,
Macquarie University

John Wasson,
University Of California Los Angeles

Richard Webb,
University Of California Los Angeles

Alycia Weinberger,
University Of California Los Angeles

Benjamin Zuckerman,
University Of California Los Angeles

Roadmap
Objectives

- #1 Sources of Organics on Earth
- #4 Genomic Clues to Evolution
- #5 Linking Planetary & Biological Evolution
- #15 Earth's Future Habitability

site. Analysis of these data and proposals for new experiments for future missions will be focused on both the search for evidence of life and the search for environments capable of sustaining life. Obvious targets are the subterranean volatiles, the chemostratigraphic and isotopic history of the polar water and CO₂ ice caps, and secular effects of orbital dynamics on volatile budgets, as well as volatile-bearing minerals and other potential biomarkers in Martian rocks. *In situ* measurements on the surface of Mars will be the basis for the selection of samples to be returned to Earth for more detailed analysis using state-of-the-art techniques. Conducting a scientific investigation on this scale requires carefully coordinated efforts among scientists from many disciplines and institutions. The collaborative environment that will be developed by NASA's Astrobiology Institute will contribute significantly toward laying the groundwork for future coordinated efforts. UCLA wishes to contribute to this endeavor.

Project

Astrobiology at UCLA: An Integrated Multidisciplinary Approach to Research and Education

Senior Project Investigator(s):
Bruce N. Runnegar, T. Mark Harrison,
James A. Lake, Benjamin Zuckerman

ACCOMPLISHMENTS

Research at UCLA is now focused on six main themes: (1) extrasolar planetary systems; (2) geobiology and geochemistry of early Earth and Mars; (3) evolution of Earth's early life; (4) genomic evolution and the tree of life; (5) celestial influences on the terrestrial environment; and (6) exploration for life in the solar system. Over the past year, some of these themes have grown significantly. The thrust of the last theme listed above has been seriously compromised by successive losses of both the Mars Climate Orbiter and the Mars Polar Lander.

Astronomy

Recent technical advances and discoveries have paved the way for an exciting new era in the detection and characterization of extrasolar stellar systems. UCLA astronomers have been deeply involved in these advances by making infrared observations of the dust, disks, and substellar companions of nearby young stars (Schneider et al., 1999; Wood et al., 1999; Telesco et al., 2000; Weinberger et al., 1999). New techniques include: (1) NIRSPEC (near infrared spectrometer) – constructed at UCLA – at the Keck Observatory in Hawaii; (2) the high-altitude SOFIA (Stratospheric Observatory for Infrared Astronomy), which will make observations in the mid- and far-infrared; and (3) the adaptive optics (AO) capability the Keck II telescope (Wizinowich et al., 2000). These instruments and the Hubble Space Telescope will be used to observe nearby clusters of young stars such as the TW Hydrae and Tucanae Associations, discovered recently (Webb et al., 1999; Zuckerman and Webb, in press). This work has already led to the detection of a faint object that may prove to be a substellar companion, which is 1-2 times the mass of Jupiter (Lowrance et al., 1999). If this is confirmed, it will be the first extra-solar planet to have been directly imaged.

Geobiology and Geochemistry

A pioneering ion probe study of the carbon isotopic composition of *in situ* Precambrian microfossils will be published soon (House et al., in press). Standards are now being developed for an in-depth study of Proterozoic acritarchs and other organically-preserved microfossils. In parallel with this work, the nature of the Hadean and early Archean environments is being explored using geochemical tracers (such as oxygen isotopes in ancient zircon crystals) and the four isotopes of sulfur (^{32}S , ^{33}S , ^{34}S , ^{36}S) in Archean and older sulfides and sulfates. (See the UCLA Field Expeditions section.) In future, this research will attempt to document the evolution of oxygen and sulfur isotopes in open ocean systems through the Archean, with a view toward obtaining information about atmospheric composition, sea water chemistry, and surface temperatures.

Paleobiology

Research is underway on Early Archean chert samples that are expected to contain microfossils. (See the UCLA Field Expeditions section.) Conical stromatolites from the same sedimentary sequences have been studied by X-ray computerized tomography in order to test numerical models for stromatolite growth being developed at UCLA. Research on ancient black smoker deposits associated with massive sulfide ores in deep-ocean environments have revealed a wealth of new fossils, including numerous vestimentiferan tube worms (Little and Cann, 1999; Little et al., 1999). Future work will focus on the microbial fossils associated with these Phanerozoic occurrences and a search for similar deposits in deep Precambrian time. Other discoveries in paleobiology include work on the importance of microbial mats in the stabilization of sands and the preservation of Precambrian soft-bodied organisms (Gehling, 1999, 2000), plus the role of trace fossils in the initial stages of the Cambrian Explosion (Jensen et al., 2000).

Genomics and Evolution

Achievements include the publication of a robust tree of life based on the protein-coding genes from a suite of microbial genomes, including yeast (Fitz-Gibbon and House, 1999). This massive analysis demonstrated that lateral gene transfer has not obliterated the phylogenetic signal in genome sequences, and it provided strong support for the broad architecture of the rRNA tree. These issues will now be explored in depth by incorporating many additional genomes into the study and by using other methods of comparative sequence analysis. Understanding the degree and effects of lateral gene transfer is a central goal of the UCLA/MBL collaboration within NAI's Evolutionary Genomics Focus Group.

Continuing inter-team research in microbial genomics and gene expression is discussed elsewhere. UCLA strongly supports the GEOPULSE initiative of the Penn State Astrobiology Research Center and will be contributing substantial resources to further development of the UCLA component of that partnership. Within the Evolutionary Genomics Focus Group, planned research on early metazoan evolution will deal with the relationships of the most deeply branching metazoan groups – sponges, cnidarians, ctenophores – and their protistan relatives.

It has long been thought that the most deeply branching living eukaryotes, such as *Giardia*, lack organelles (mitochondria and chloroplasts) because they never had them.

Recent work has shown that many of these amitochondriate protists have nuclear genes that have a mitochondrial origin and, in some cases, a mitochondrial function (e.g., Roger, 1999). At the same time, it has become clear that organelles known as hydrogenosomes may be best explained as mitochondrion-like bodies that have lost all their genes. Both of these ideas need to be tested because they have important implications for understanding the early history of eukaryotes and the way in which the eukaryote genome evolved. This work will be carried out at UCLA and at Dalhousie University using several recently discovered and hydrogenosome-bearing anaerobic protists that are in culture at UCLA.

Planetary Science

Two areas of great interest to astrobiology are the effects of major impacts on the history of life and the effects of secular changes in Earth's orbital parameters on climate and the evolution of the biosphere. The first is being investigated through the discovery and characterization of impact deposits in undisturbed sedimentary sequences. For example, thick spherule beds with anomalous iridium and chromium isotope ratios provide evidence for the frequency of devastating impacts in early Archean times (Anbar et al., in press; Shukolyukov et al., in press). In the second case, long numerical integrations of solar system dynamics are being tested through the stratigraphic record of distinctive climatic events that are reflected in global changes in ocean chemistry. An unexpected outcome of this research has been the discovery of the sensitivity of orbital calculations to the ways in which some components of the system are approximated. As a result, we are rewriting the codes from first principles and plan to test and apply the results of long numerical integrations to problems on both Earth and Mars.

Solar System Exploration

The sequential loss of the Mars Climate Orbiter (MCO), then the Mars Polar Lander (MPL), has seriously disrupted the UCLA program in astrobiology. However, the operations facility, which was constructed in the Science and Technology Research Building (STRB) for the MPL mission, and the core personnel to run that facility are both resources that should not be squandered at this stage. We are supporting this endeavor by providing modest bridging funds to: (1) allow graduate students who were brought on board to work on data from the MCO and MPL missions to develop new research projects; and (2) help with a transition to new experiments and missions. Planned work on MCO images of the polar layered deposits will combine image analysis with numerical studies of the eccentricity and obliquity of Mars.

HIGHLIGHTS

- Robust whole-genome tree of life (Fitz-Gibbon & House, 1999)
- Discovery of seven T Tauri stars and a brown dwarf in the nearby TW Hydrae Association (Webb et al., 1999)
- Biochemical evidence for the common ancestry of mitochondria and hydrogenosomes (Dyall et al., 2000)
- Microbial mats are the key to Ediacaran preservation (Gehling, 1999)

- Confirmation of early Archean giant impact deposits using chromium isotopes (Shukolyukov et al., in press)
- Adaptive Optics (AO) comes on line at the Keck Observatory (Wizinowich et al., 2000)
- Carbon isotope compositions of individual Precambrian microfossils (House et al., in press)
- Homeobox gene engrailed is implicated in skeleton formation (Jacobs et al., in press)
- The first extrasolar planet may have been observed (work in progress)
- Horizontal gene transfer limited by complexity of gene product interactions (Jain et al., 1999; Lake et al., 2000)
- Namibian trace fossil record ruins Precambrian-Cambrian boundary definition (Jensen et al., 2000)
- Hydrothermal vent communities traced back to Paleozoic (Little et al., 1999; Shpanskaya et al., 2000)
- Planetary atmospheres are not significantly eroded by giant impacts (Newman et al., 1999)
- Spectacular circumstellar disk imaged with NICMOS (Near-Infrared Camera and Multiobject Spectrometer) (Weinberger et al., 1999)

Field Expeditions

Purpose: Oldest known terrestrial materials: 4+ billion-year old zircons

Location: *Yilgarn Craton, Western Australia*

Dates: June 22 to July 14, 1999

Participants: *T. Mark Harrison, University of California, Los Angeles, *Stephen Mojzsis, University of California, Los Angeles, R.T. Pigeon, Curtin University of Technology, Perth

The Narryer Gneiss Complex is a fragment of Early Archean crust that is composed of metamorphosed sedimentary rocks. Some of these metasediments contain zircons that have U-Pb ages >4 Ga. These tend to be very rare so the field work in 1999 focused on quartz-pebble conglomerates rich in heavy mineral indicators such as chromium spinel. A newly developed multi-collection ion probe technique was then used to survey several hundred zircon grains obtained from the field samples and several grains with ages up to 4,280 Ma were discovered. These are being analyzed for chemical evidence of Earth's earliest surface environments. A preliminary report of this research was given by Stephen Mojzsis in an invited oral presentation at the First Astrobiology Science Conference, NASA Ames Research Center, April 2000.

Purpose: Archean stromatolites, microfossils, and environments

Location: Pilbara, northwestern Western Australia

Dates: June 29 to July 6, 1999

Logistics: *Geological Survey of Western Australia*

Participants: Stanley Awramik, University of California, Santa Barbara; *David Des Marais, NASA Ames Research Center, *Jack Farmer, Arizona State University; Andrew Glikson, Australian National University; Kathleen Grey, Geological Survey of Western Australia; Arthur Hickman, Geological Survey of Western Australia; Hans Hofmann, University of Montreal; *Bruce Runnegar, University of California, Los Angeles; *J. William Schopf, University of California, Los Angeles; Mike Sommers, University of California, Santa Barbara; Martin Van Kranendonk, Geological Survey of Western Australia; Malcolm Walter, Macquarie University; Ian Williams, Geological Survey of Western Australia

Motivation for this field trip was to bring experienced Precambrian paleobiologists to a recently discovered site that has well-preserved conical stromatolites about 3.5 Ga old. Geologists at the Geological Survey of Western Australia who had discovered the site wanted it documented before removing some of the best specimens for display in Perth at the Western Australian Museum. In addition, the event was used for educational purposes, both through a public seminar held during the field trip in the remote town of Marble Bar and by having the Australian Broadcast Corporation's media crew shoot a segment on the Archean stromatolites for the highly popular Australian TV science program "Quantum". Members of the NAI were central participants in both events.

UCLA participants (Runnegar and Schopf) collected five drums of samples, mainly black cherts, silicified stromatolites, and sedimentary barites. Schopf has had numerous chert thin sections prepared and will be using novel analytical techniques (ion microprobe analysis, Raman spectroscopy, and microscale computerized tomography) to obtain metabolic as well as morphological information from any microfossils that are discovered. Runnegar has used X-ray computerized tomography and other techniques to characterize the mineralogy of the sedimentary barites. He is also involved in a collaboration that is producing exciting new evidence from sulfur isotopes about the nature of the Early Archean environment (Farquhar et al., in press). Runnegar and Per Jögi are using stromatolites collected during the field work as the basis for testing new mathematical modes of stromatolite growth.

Purpose: Cretaceous hydrothermal vent communities in the Neotethyan ocean

Location: *Troodos Ophiolite, Cyprus*

Dates: April 16 to 29, 2000

Logistics: Department of Earth Sciences, University of Leeds, U.K.

Participants: Andrew Barnicott, University of Leeds; Joe Cann, University of Leeds; *Crispin Little, University of California, Los Angeles

Hydrothermal vent communities are rare in the fossil record, but the Troodos Ophiolite preserves an unprecedented number of examples. The Troodos Ophiolite is an exceptionally preserved piece of ocean crust of late Cretaceous age now sandwiched between the European and African plates and exposed on the island of Cyprus. A previous field trip to Cyprus in 1997 found vent fossils in six massive sulfide deposits in the ophiolite, comprising vestimentiferan tube worms and various gastropod mollusc species

(Little et al., 1999). Field work in 2000 aimed to expand the number of fossil species and specimens from those massive sulfides already searched. This resulted in the collection of large number of new samples currently being studied.

Cross Team Collaborations

UCLA has been involved in the founding and development of the following NAI Focus Groups and cross-team activities. As the Focus Groups develop and hold their first workshops, UCLA's involvement in NAI collaborations is expected to increase significantly.

Ecogenomics Focus Group

UCLA has a significant interest in microbial ecology (described below), which is complementary to the main thrust of the Ecogenomics Focus Group.

Evolutionary Genomics Focus Group

This group was co-founded by S. Blair Hedges (Penn State) and James A. Lake (UCLA). Its goals are well articulated in a proposal submitted under the CAN augmentation opportunity. If the proposed research is funded, NAI members from Caltech/JPL (Joseph L. Kirschvink), Harvard (Charles R. Marshall), MBL (Monica Riley), Penn State (S. Blair Hedges), UCLA (Jacobs, Lake, Runnegar), and the University of Florida (Steven A. Benner), plus their postdocs and graduate students will be working together in an unprecedented nation-wide collaboration.

Geomicrobiology and Microbial Genomics

Christopher House (Penn State) and Sorel Fitz-Gibbon (UCLA) are maintaining and developing close collaboration, which they began at UCLA. Proposals now pending are expected to fund studies that focus on the expression of metabolic genes with environmental implications in two archeal thermophiles with completely sequenced genomes: *Pyrobaculum aerophilum* and *Methanosarcina thermophila*. This work will be done in conjunction with Jeffrey H. Miller (UCLA) and James G. Ferry (Penn State), and it follows from on-going collaboration with Karl O. Stetter (University of Regensburg/UCLA).

Mars Focus Group

UCLA (Runnegar, Paige, and Schopf) assisted with preparation of the Mars Program Architecture report to NASA Headquarters in February, 2000. Our UCLA group is now deeply involved in restructuring the Mars Polar Lander facility in UCLA's Science and Technology Research Building for future opportunities in Mars exploration. We expect to work closely with other NAI teams, especially Arizona State, as the Mars program develops.

Proposed Mars Meteorite Focus Group

UCLA has a longstanding interest and considerable experience with Mars meteorites, culminating in research on "Los Angeles," the most recently identified Martian meteorite. We support the concept of a Mars Meteorite Focus Group and expect to play an active role within such a group

Proposed Europa Focus Group

UCLA has a significant contribution to make to this proposed focus group through expertise in magnetospheric physics and dynamical modeling (e.g., Pappalardo et al.,

1999). These are the fields that currently provide the most useful information about the nature of the ice/ocean on Europa. We envisage playing a significant role in future activities of this proposed group.

Proposed Impact Histories Focus Group

This is a UCLA initiative that will be explored by Frank Kyte during planned summer consultation with the international community.

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PUBLICATIONS & ABSTRACTS

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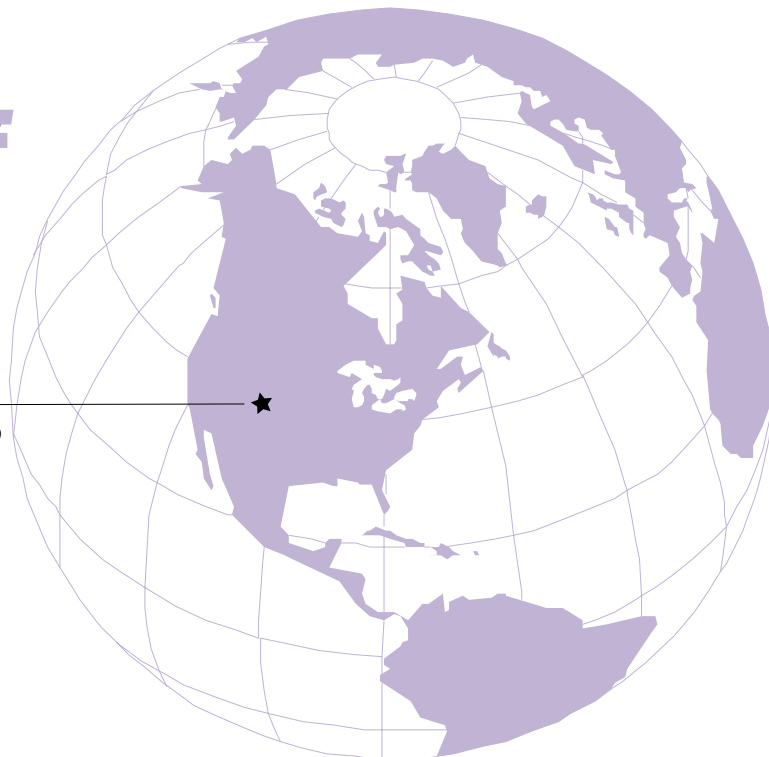
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UNIVERSITY OF COLORADO, BOULDER

Boulder, Colorado



PRINCIPAL INVESTIGATOR



**Bruce
Jakosky**

UCO TEAM MEMBERS

John Bally,

University Of Colorado Boulder

Erica Barth,

University Of Colorado Boulder

Carrine Blank,

University Of Colorado Boulder

Jason Bobe,

University Of Colorado Boulder

Sheralee Brindell,

University Of Colorado Boulder

Carole Cleland,

University Of Colorado Boulder

Tony Colaprete,

University Of Colorado Boulder

Dan Curtis,

University Of Colorado Boulder

Scott Dawson,

University of California Berkeley

Jose de la Torre,

University of California Berkeley

Eric DeChaine,

University Of Colorado Boulder

Sarah Earley,

University Of Colorado Boulder

PROPOSAL EXECUTIVE SUMMARY (1998)

The University of Colorado at Boulder proposes to create a Center for Astrobiology (CAB) that will serve as a focal point, both for research in astrobiology already taking place at the University and for new research that would be supported through this proposal. The intellectual breadth will span in depth the parts of astrobiology relating to: (1) the origin and evolution of life on Earth; (2) the environmental conditions necessary for the existence of life; (3) the nature, habitability, and potential for life on the planets in our solar system; (4) the formation of planets around other stars and the potential they hold for life; and (5) the philosophical and religious significance of searching for life elsewhere.

Ongoing research in astrobiology is taking place separately in several of the component disciplines and in a number of different academic units on campus. The University of Colorado is internationally recognized for its research in the formation of stars, the origin of life and the RNA world, the evolution of life, as well as the nature, evolution, and habitability of planets. Research in these areas is spread through a number of academic departments and research laboratories that span the physical, biological, and chemical fields. There are 25 members of the faculty at CU who will participate in the activities of the CAB. Eighteen of these are tenured or tenure-track faculty, and the rest are in research positions supported by research grants. In addition, two members of the nearby Southwest Research Institute and one scientist from Lockheed-Martin Astronautics in Denver will participate. The proposed Center for Astrobiology will allow us to coordinate the diverse activities and obtain the benefits of synergy by bringing them together.

New research by existing faculty members will be supported by this proposal, covering the entire breadth of topics encompassed in understanding the nature of life in the universe and focusing on understanding the broad scientific issues related to life in the universe.

Specific research topics for the University of Colorado at Boulder will include the six following areas.

Year 2

Visit our site

<http://argyre.colorado.edu/life/CAB.html>

- *Formation of Stars and Planets*

We will conduct an observing and analysis program using a variety of ground- and space-based platforms to understand the degree to which protoplanetary disks will evolve into debris disks that can accrete to form planets before they are destroyed by radiation fields, stellar winds, and explosions of nearby stars.

- *Habitability of Planets*

We will investigate the global effects of clouds and hazes on planetary climate and habitability, particularly the behavior of clouds created by impacts onto the Earth and other planets

- *Origin of Life and the RNA World*

We will develop an alternative means of searching for chemical activity in nucleic acid sequences (as in a primitive RNA environment) using sensitive direct chemical assays rather than biochemical selection. With this new approach, we expect that new activities might be discovered and that this should lead to new ideas about the appearance and rise to prevalence of rare RNA activities in a primitive RNA world.

- *Evolution of Life on Earth*

We will study a suite of structural characteristics associated with the transition of photosynthetic organisms from water to a terrestrial environment, as a means of studying paleobiological and evolutionary roles of photosynthetic organisms and the pattern of life and general features of key habitat transitions characterizing life on Earth throughout its history.

- *Energetics of Life on Other Planets*

We will explore the availability and accessibility of geochemical energy from geological environments on Mars, the early Earth, and possible Earth-like planets elsewhere, all with specific attention to hydrothermal systems and to weathering of rocks as a means of obtaining energy to sustain life prior to the onset of photosynthesis.

- *Philosophical Implications*

We will look at philosophical implications of the search for life elsewhere and the interactions between the scientific results and society. We will explore the questions of what constitutes life and what would constitute "good" evidence that life existed in an extra-terrestrial system, all in the broader context of the history and philosophy of science.

New research also will be carried out by two new faculty members to be hired by the University and supported as part of this proposal. The two positions are in key areas of astrobiology and fill gaps in the existing program at Colorado University. One will be in the Department of Geological Sciences and will work in the area of understanding the

William Friedman,
University Of Colorado Boulder

Dave Glandorf,
University Of Colorado Boulder

Kirk Harris,
University Of Colorado Boulder

Vasant Jadhav,
University Of Colorado Boulder

Bruce Jakosky,
University Of Colorado Boulder

Elinor Newman,
University Of Colorado Boulder

Norman Pace,
University Of Colorado Boulder

Bo Reipurth,
University Of Colorado Boulder

Cristina Rumbaitis-del Rio,
University Of Colorado Boulder

Teresa Segura,
University Of Colorado Boulder

John Spear,
University Of Colorado Boulder

Henry Throop,
University Of Colorado Boulder

Margaret Tolbert,
University Of Colorado Boulder

Owen Toon,
University Of Colorado Boulder

Stacy Varnes,
University Of Colorado Boulder

Jeffrey Walker,

University Of Colorado Boulder

Michael Yarus,

University Of Colorado Boulder

geological evidence and constraints pertaining to the early history of life on Earth. The second will be in the Department of Molecular, Cellular, and Developmental Biology and will work in the area of the microbiology of prokaryotic organisms that are relevant to the early nature and history of life on Earth.

Specific research to be carried out by our two new faculty members will be: (1) The geologic record of early life on Earth. We will explore the Earth's geologic record in order to understand better the earliest history of life on Earth and the physical and chemical environments in which it existed. (2) Microbiology of prokaryotic organisms. We will explore the nature of prokaryotic organisms on the Earth, focusing on both modern-day organisms and fossil cells, in order to understand environmental limits on their existence, the processes by which they metabolize, and their evolution.

With our strengths in the entire breadth of understanding the nature and distribution of life in the universe, we will be able to offer a broad course of study in astrobiology at both the undergraduate and graduate levels. The goals will be to train the next generation of interdisciplinary scientists to work in the field of astrobiology and to educate the general public about the scientific, technical, and societal aspects of the search for life elsewhere. By fostering world-class research and teaching in the broadest aspects of the field of astrobiology, we will be able to offer an integrated program of study that is self-contained at the University of Colorado and the town of Boulder.

We also will offer a broad program of outreach at all levels, including outreach to other related University programs, to the broad intellectual community (including members of the University and the local community), to the larger public, and to teachers and students involved in K-12 education. The goal of the outreach will be to enhance the educational and intellectual environment in astrobiology and to bring the results of our research program back to the citizenry as a whole.

In addition to having a self-contained research, teaching, and outreach program, we will participate in the NASA Astrobiology Institute (NAI), and will make use of the Next Generation Internet (NGI) to enhance the value obtained. Use of the NGI will enable communication between computers (and enhancement of the ability to do science that goes with it), communication between individual scientists and between small groups of scientists (to facilitate interaction between related research tasks at the various institutions that are a part of the NAI), and group communication involving colloquia, workshops, and courses (that will foster broad-scale communication, now typically possible only once or twice a year when a limited number of people can travel to participate on-site in these types of activities).

The University of Colorado has made a substantial commitment to astrobiology in the past by developing world-class programs in many of the individual component disciplines in astrobiology. In addition, substantial new commitments will be made to support the present proposal. These include creation of a new Center for Astrobiology, two new faculty positions (as described above), a total of \$200K in matching funds on equipment to outfit laboratories of the new faculty members, a contribution from LASP (UC Laboratory for Atmospheric and Space Physics) of \$150K over five years to support activities of the Center, teaching assistant support for undergraduate courses in astro-

Year 2

biology, and a substantial contribution of resources of the existing faculty members who will take part in the CAB.

The Center for Astrobiology will coordinate research and teaching activities of its members in order to provide a coherent research program and learning environment in astrobiology. Additionally, it will foster activities in astrobiology such as colloquia, seminars, workshops, and a visiting scholars program, as well as support the astrobiology program through a small discretionary fund and through coordination and oversight of NGI activities.

In summary, we are able to offer NASA a large program in astrobiology. Our program will integrate well with and provide leadership for the nationwide effort, all for the cost of a small-to-moderate sized program.

Origin and Early Evolution of Terrestrial Photosynthetic Life

Project

Senior Project Investigator(s):
Bruce Jakosky

ACCOMPLISHMENTS

During our work this past year, two aspects of the origin of terrestrial photosynthetic life have been studied: (1) the origin of water conducting cells to allow autotrophic life to move from water to land; and (2) the origin of symbiosis as a key to the colonization of terrestrial environments. Our work on symbiosis involves an investigation of the mycorrhizal associations between primitive land plants and specific fungi that are hypothesized to have been critical to the initial colonization of land by photosynthetic green algae. We are using molecular markers to determine the specific fungi that engage in symbiotic relationships with primitive plants. In the project involving study of the ability of photosynthetic organisms to translocate water within increasingly complex bodies, we have made major progress in interpreting some of the earliest fossil records of water conducting cells (Silurian and Devonian). This work will appear in the *Philosophical Transactions of the Royal Society* in 2000. During the next year, the main focus will be on the evolution of plant fungal symbiosis and its bearing on the colonization of terrestrial environments.

Roadmap Objectives

#6

Microbial Ecology

#10

Natural migration of Life

Roadmap Objectives

#8

Past & Present Life on Mars

Project

Geochemical Weathering and the Energetics to Support Life on Mars

Senior Project Investigator(s):

Bruce Jakosky

ACCOMPLISHMENTS

The primary objective of this research is to constrain the amount of chemical energy potentially available from reactions between ground or surface water and regolith or crustal rock to support life on Mars. To accomplish this goal, we will integrate broad aspects of terrestrial and Martian geochemistry, geochemical computer modeling, metabolic strategies of terrestrial analog organisms, as well as an understanding of geologic processes occurring on Mars.

Our approach is to use the standard geochemical software tools EQ3/6 (modeling software) and SUPCRT92 (interactive database and software programs). We require particular knowledge of a number of environmental parameters as inputs into the models, including the chemical composition of crustal rocks and groundwater, the volume of material chemically altered, and the composition of the atmosphere in contact with groundwater.

The sites to be modeled are still under consideration, but they will include the "hematite" deposit in Sinus Meridiani. This particular site will be modeled both as an area of low-temperature water/rock interaction as well as an area of hydrothermal alteration. Progress to date includes importing the EQ3/6 and SUPCRT92 packages, understanding their appropriate use and theoretical underpinnings, and developing a small pilot study to determine the amount of chemical energy available from the interaction of a SNC composition meteorite with hydrothermal fluids.

Roadmap Objectives

#18

Currently this project does not fit in a current category

Project

Societal and Philosophical Aspects of Astrobiology

Senior Project Investigator(s):

Bruce Jakosky

ACCOMPLISHMENTS

We are working to understand the societal issues of astrobiology (such as why the country is so interested in the search for life, despite the lack of practical relevance) and to help the astrobiology community appreciate the value of addressing these questions. Our approach is first to describe the broader nature of astrobiology and planetary exploration in our society today and how the public interacts with these fields. Next, we

explore what the philosophical drivers are behind the interactions.

Our goal is to understand better the role of science in society, especially the role that scientists can play in those interactions. So far, our efforts have resulted in two publications, one in *Proceedings of the 1999 Bioastronomy Conference* and one in *EOS*, the weekly newspaper of the American Geophysical Union. (See citation below.) Additionally, we have given about a dozen public talks in the last year, including numerous ones around the country. Our numerous interactions with the broadcast and print media have been discussions on aspects of planetary science and astrobiology. Ongoing activities include work on an "edited by" book on the societal connections of astrobiology and on a sole-author book on the same topic.

HIGHLIGHTS

- Published article in *EOS, Transactions of the American Geophysical Union* on astrobiology and society. This is the first position paper published on the topic, and it appeared in a place that reaches about 40,000 scientists.
- Jakosky, B.M. & Golombek, M.P. (2000). Planetary science, astrobiology, and the role of science and exploration in society. *EOS, Transactions of the American Geophysical Union*, 81(6) 8 February: 58.

The Formation of Planets Around Young Stars

Project

Senior Project Investigator(s):
John Bally

ACCOMPLISHMENTS

Our main research results from the past year are organized below into five sections, all relating to the overall topic of planet formation around young stars.

Grain Growth in Protoplanetary Disks

We have discovered evidence for grain growth in protoplanetary disks surrounding young stars embedded within the Orion Nebula. Modeling of the disk properties indicates that the mean particle sizes are at least several micrometers, and a fraction of the mass in these disks may be locked up in particles larger than 1.3 mm. Grains may grow to such large sizes within less than 100,000 years.

Star and Protoplanet Formation

The majority of stars appear to form in Orion Nebula-like environments in OB star associations where young planetary systems face many hazards. Protoplanet formation must be prompt, or exo-planets will be rare. Within the past 10 Myr, about 20,000 to 50,000 stars were born in OB associations within 500 pc of the Sun. Within the same region, only about 5,000 to 10,000 stars were born in shielded dark clouds. Thus, between 70% to over 90% of all stars are born in OB associations.

Roadmap Objectives

#11

Origin of Habitable Planets

Roadmap Objectives

#18

Currently this project does not fit in a current category

Protoplanetary Systems

We have started to constrain the possible architectures of exo-planetary systems. Protoplanetary systems forming in Orion Nebula-type environments rapidly lose the volatile (H, He, CO, etc.) and small grain components of their disks due to photo-ablation. Thus, gas giants are not likely to form in Orion-like environments unless they do so within the few hundred thousand years prior to the onset of external UV irradiation. However, enough material may already be locked into large bodies so that rocky planets may eventually form around these stars.

Protoplanetary Disks

Hazards to planet formation include radiation fields which photo-ablate protoplanetary disks, and three-body stellar encounters in the dense proto-star clusters and nascent binary/triple star systems. Dynamical encounters with close companions truncate or destroy protoplanetary disks.

Project

Philosophical Significance

Senior Project Investigator(s):
Carol Cleland

ACCOMPLISHMENTS

My work has developed a new philosophical account of the difference between the methodology of historical science, which includes hypotheses in planetary science and astrophysics, and laboratory science. My account grounds the difference between these practices in an objective and pervasive feature of nature, thus defeating claims (by the likes of Henry Gee, an editor of *Nature*) that historical science is either inferior to laboratory science or not even bona fide science.

I have applied this analysis to the debate over whether ALH84001 contains fossilized Martian life. Having finished 2 papers on this topic (one for the lay person and the other for scientists), I am currently working on another paper for philosophers of science. I have achieved the goals that I set for this year. As I continue my work in this next year, I am planning to begin working on issues centering on whether there could be an adequate definition of life.

HIGHLIGHTS

- Historical science is real science.
- The difference between historical science and laboratory science: Searching for the smoking gun.
- Life in ALH84001?: Confirming the best explanation.

Year 2

Roadmap
Objectives**Molecular Analysis of Microbial Ecosystems
in Extreme Environments**

Project

Senior Project Investigator(s):
Norman R. Pace**ACCOMPLISHMENTS**

Our projects revolve around the development and use of ribosomal RNA-based (rRNA-based) molecular methods to survey and study the microbial constituents of ecosystems in extreme environments, without the requirement of cultivating these organisms. Rather than using classical methods, this cultivation-independent approach to ecosystems analysis is essential, because most of these extreme environment microbes (>99%) are not cultured using standard techniques. With the molecular methods, rRNA genes are cloned directly from environmental DNA. Then, they are sequenced to gain a phylogenetic snapshot of the organisms represented by the cloned genes. Some properties of organisms can be inferred from the phylogenetic results, and the sequences can be used to design hybridization probes to visualize organisms and their interactions in the environment.

Project study areas relevant to NAI are discussed in four groups listed below.

1. Antarctic Endolithic Communities (Collaboration with Imre Friedmann, University of Florida). Most primary productivity in Antarctica is due to endolithic microbial communities, which are photosynthesis-driven communities in the outer few centimeters of any rock surface exposed to light. These communities so far have received only limited study, with classic microscopy and culture techniques. Ongoing rRNA gene analysis of two selected communities has revealed many novel kinds of organisms, some closely related to described organisms, but others very different.

Although previously considered dominated by cyanobacteria, an abundance of chloroplast sequences has been detected. The nature of the eucaryal component expected is not yet known. Remarkably, an abundance of representatives of the *Thermus/Deinococcus* division of Bacteria has been detected. These were previously unknown, but they are related to the "radiation resistant" deinococci. These new organisms also may predictably be similarly robust. The "radiation resistant" property of the deinococci is now thought to be from dessication/oxidative damage.

2. Yellowstone High-Temperature Settings (some collaboration with Sherry Cady, U. Portland). The laboratory has for many years studied thermophilic ecosystems at Yellowstone and elsewhere. Current activities continue to explore the makeup of properties of communities driven by hydrogen-metabolism, probably the dominant form of primary productivity in high-temperature settings anywhere. A novel, field hydrogen detector will be used during this year to survey hydrogen fluxes in selected Yellowstone hot springs. An array-based culture system has been devised that allows the study in culture of particular organisms ("phylotypes") in mixed-community enrichment cultures. Fluorescence *in situ* hybridization, with probes based on rRNA gene sequences, is used

#2
Origin of Life's Cellular
Components#3
Models for Life#4
Genomic Clues to
Evolution#5
Linking Planetary &
Biological Evolution#6
Microbial Ecology#7
Extremes of Life#8
Past & Present Life on
Mars#9
Life's Precursors &
Habitats in the Outer
Solar System#10
Natural Migration of
Life#14
Ecosystem Response
to Rapid
Environmental Change#16
Bringing Life with Us
Beyond Earth#17
Planetary Protection

Roadmap Objectives

#12

Effects of Climate &
Geology on Habitability

to detect specific phylotypes in complex enrichment cultures.

3. Anaerobic environmental eucaryotes. We have been conducting an rRNA-based survey of eucaryal phylotypes in anaerobic settings, for instance anaerobic marine and freshwater sediments. Recent results have identified a wealth of novel eucaryotic microbial diversity, including seven (!) novel kingdom-level clades, some among the most deeply divergent of eucaryal rRNA sequences. Attempts to learn more about those organisms represented by the sequences are underway.

4. Application for NAI augmentation support for a study of hypersaline microbial communities has been made.

The PI participates in numerous astrobiology-related public and institutional activities, for instance service on the new Space Studies Board committee, Committee on the Origin and Evolution of Life; and organizing (with others) the recent SSB/NRC workshop report, "Size Limits of Very Small Microorganisms."

HIGHLIGHTS

- Discovery of seven new kingdom-level phylogenetic groups of eucaryotes in anaerobic environments. These include forms that branch deeply in the eucaryal phylogenetic tree. They are highly divergent from known organisms.

Field Expeditions

August, 1999, *Yellowstone National Park*: University of Colorado, Portland State University

September 1999, *Yellowstone National Park*: University of Colorado

October, 1999, *Yellowstone National Park*: University of Colorado

December, 1999, *Yellowstone National Park*: University of Colorado

Project

Factors That Influence Long-Term Habitability of Planets: Effects of Clouds and Impacts on Planetary Environments

Senior Project Investigator(s):
Owen B. Toon

ACCOMPLISHMENTS

The goal of our research is to understand the factors that influence the long-term habitability of planets. O.B. Toon's work is partly sponsored through the University of Colorado Astrobiology Program. Margaret Tolbert's work and part of O. B. Toon's work is sponsored by the NASA Astrobiology Institute. In this latter work, we are interacting with the climate-modeling group at NASA Ames Research Center and Professor Jim Kasting of Pennsylvania State University, who is also a member of the NASA Astrobiology Institute. This report summarizes both projects since they are highly interactive.

Year 2

Our first project is to understand the role of clouds in planetary climates. We are using laboratory facilities in Dr. Tolbert's group to guide theoretical modeling of clouds on Mars and clouds on Titan. David Glandorf, a graduate student in the Chemistry Department, has finished experiments that show how carbon dioxide condenses onto ice surfaces. This work provides critical information that is needed to model carbon dioxide clouds in early atmospheres of Mars (and possibly Earth), as well as in the current atmosphere of Mars. We have submitted a paper on this work to *Geophysical Research Letters*. Current spacecraft observations of Martian clouds have been used to test our models that incorporate the laboratory work mentioned earlier. Tony Colaprete, a graduate student in the Astrophysical and Planetary Science Department, has simulated the clouds that may have been present in these early atmospheres, as well as in the current Mars atmosphere. We find that we can reproduce the current cloud properties as observed from Mars Observer. We also find that clouds in early atmospheres tended to cool the planet (not warm it), but they don't totally cancel the greenhouse effect of added carbon dioxide gas.

Another cloud-related project focuses on understanding the clouds of Titan. Dan Curtis, a graduate student in the Chemistry Department, is investigating how methane and ethane will condense on the organic hazes thought to exist in the atmosphere of Titan. Similar materials may have once been abundant in Earth's atmosphere. Our goal is to understand if clouds are likely to form in Titan's atmosphere, or whether supersaturated vapors are more likely. We plan to use these laboratory studies to guide a theoretical model that Erica Barth is developing. To date, we find that episodes of ethane cloud formation may occur every few hundred days on Titan, with ethane drizzle every few months. We will compare the model with Titan observations, those currently available and also those planned for the Cassini mission in a few years.

Our final project is to understand the role that impacts play in planetary environments. We have previously done extensive studies of the environmental effects of impacts on Earth, work which Elinor Newman is continuing. Now, we are trying to determine if the rivers on Mars may have been caused by impact events on the surface, rather than by greenhouse warming as most people currently think. Teresa Segura, a graduate student in the Atmospheric and Oceanic Science Department, has been conducting modeling studies of the Martian atmosphere after a large impact. She plans to spend the summer at NASA Ames Research Center consulting with Kevin Zahnle and Bob Haberle on this project.

HIGHLIGHTS

- Laboratory studies constrain models of cloud formation on Mars.
- Our resulting model (from laboratory studies and data) can reproduce the features of clouds observed on current Mars from Mars Observer.
- Models show that previous theories (stating that carbon dioxide clouds on early Mars would contribute to the greenhouse effect) are incorrect.

Roadmap Objectives

#2

Origin of Life's Cellular Components

#3

Models for Life

Project

Laboratory Study to Detect RNA Activities to Test the RNA World Hypothesis

Senior Project Investigator(s):

Michael Yarus

ACCOMPLISHMENTS

We have begun working on a highly sensitive method for detection of new RNA activities. The detection method, DICE (differentiation control element), will be of use in evaluation of the RNA world hypothesis. In particular, DICE should complement the present means available, with its selection-amplification for isolation of rare catalytic activities in large randomized ribonucleotide sequence pools.

This year, we have acquired fluorogenic substrates suitable for DICE, based on a search of the literature. The substrates, intended for isolation of RNA proteases, have been tested with a model catalyst, highly purified trypsin. We have confirmed that: (1) these molecules are stable in the absence of specific catalysis; (2) their fluorescence has the favorable low background reported in the literature; and (3) they are readily hydrolyzed by the protein. These substrates thus seem suitable for the next stage of the project, which is detection of activity using instrumentation suited to surveys of many randomized RNA molecules simultaneously.

HIGHLIGHTS

- Research progress in exploring new methods for isolating unusual catalytic RNAs and thereby testing the RNA world hypothesis

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Focus Groups

Year 2

Focus Group

Mars

Chair(s):
Jack Farmer

The NAI Mars Focus Group was formed to: (1) provide a regular forum for discussion of important scientific goals and objectives for the astrobiological exploration of Mars and (2) offer recommendations for implementing missions to Mars Program planners. Initial and ongoing activities focus on the selection of targets for orbital remote sensing and for *in situ* exploration by landed missions. Last year the Focus Group conducted several video conferences to review options for landing sites for astrobiology, then prepared a white paper recommendation to assist the NASA Mars mission planning community in re-formulating a new Mars Program architecture. These recommendations were presented to the Mars Architecture Team at JPL (headed by Charles Elachi) and disseminated in published form to a broad spectrum of the Mars planning community.

Future activities will broaden membership of the group to include scientists outside of the NAI and return to our discussions of science objectives and Mars landing sites for future missions. This will involve videocon-based reviews of high priority sites, followed by discussions focused on the scientific potential of each site, and recommendations for implementation. Group discussions will aim to achieve a consensus regarding site prioritization and science implementation. To enhance site accessibility for any given mission, recommendations will be presented regularly to mission planners and engineers through existing Mars planning committees (e.g., Mars Exploration Payload Advisory Group (MEPAG); The Site Selection Steering Committee; The Astrobiology Task Force). Results of site reviews and discussions will be archived, along with supporting imaging and other data from Viking and MGS (Mars Global Surveyor), on the Center for Mars Exploration (CMEX) website that will be updated as new information is obtained (<http://cmex-www.arc.nasa.gov/>).

Year 2

Ecogenomics

Focus
GroupSenior Project Investigator(s):
Mitchell Sogin, David Des Marais

PROPOSED RESEARCH

"Where did we come from, how did we get here, and where are we going?" embodies the principle objectives of astrobiological research. Answers to the first question are rooted within the microbial world, which represented the only form of life during the initial three billion years of our evolutionary history. Ever since the origin of life, complex interactions of microorganisms with each other and with all other components of the biosphere have dominated the course of evolution in our biosphere. Even the earliest biogenic fossils (microbial mats) display integrally layered patterns of organization. This spatial structuring likely reflects functional interdependence of different microbes in early communities.

Microbial creatures of untold diversity continue to dominate every corner of our biosphere, and they are likely to be the only life forms that might be encountered in other parts of our solar system, if not the entire cosmos. Yet, there is only sparse information about the true diversity of microorganisms, including their capability to orchestrate and drive key biogeochemical cycles that shape our ever-changing planet. A more complete understanding of microbial diversity, descriptions of ecosystem-wide patterns of gene expression, and detailed analyses of biogeochemistry would provide a new foundation for interpreting paleontological and geological studies that describe Earth's early history. The NASA Astrobiology Institute (NAI) may be uniquely positioned to initiate an interdisciplinary project to address these questions.

Molecular techniques similar to those employed by many teams in the NAI allow us to identify all kinds and numbers of microorganisms in a given environment without requiring their cultivation in the laboratory. Such molecular surveys have revealed new levels of largely-unexplored microbial diversity, both in geothermal environments and in the cold oceans. Yet, this technology has been applied to only a handful of environments, with few being directly relevant to conditions that supported the earliest forms of life. Furthermore, we also have only a superficial understanding of how to correlate particular members of a dynamic microbial ecosystem with the wide array of biogeochemical activities and underlying patterns of gene expression.

With modern technology developed by the genome community, we can assess microbial diversity and the total genetic coding capacity of any particular environment. This would be based on phylogenetic surveys of ribosomal RNAs and high-throughput DNA sequencing. The following is a bold strategy for linking microbial gene expression patterns with particular metabolic activities that underlie central biogeochemical processes. Community DNA extracted from natural microbial populations of a selected site will be treated as a complex mixed genome. Members of the microbial community will be

Roadmap
Objectives

#3

Models for Life

#4

Genomic Clues to
Evolution

#5

Linking Planetary &
Biological Evolution

#6

Microbial Ecology

#7

Extremes of Life

surveyed by analysis of rRNAs, and descriptions of potential metabolic diversity will be inferred from database analyses of several hundred thousand randomly selected DNA sequences. This large database of DNA sequences from the "mixed environmental genome" will be used to design DNA microarrays that can detect mRNA transcription patterns. With this mixed environmental genome array technology (MEGAT), tens of thousands of distinct genes of known sequence and function can be efficiently monitored. MEGAT will define changes in gene expression from a large number of environmental samples with known spatial and temporal distribution patterns in a well-structured microbial community, e.g., microbial mats or stromatolites. Results of these studies will be correlated with detailed measurements of biogeochemical gradients throughout the studied environment. The initial objective is to define biological complexity within the studied microbial system. The ultimate objective is to model how coordinated gene expression patterns observed in microbial consortia shape the environment.

The project would be pursued as a collaboration between many, if not all, of the astrobiology research teams. Its activities would be supported by supplemental funding from the NAI. Resources will be needed to support fieldtrips to collect nucleic acid samples and coordinately measure biogeochemical parameters. A budget must be established for high-throughput DNA sequencing by one of the astrobiology teams or by an appropriate subcontractor. It will also be necessary to assemble and support a DNA microarray facility. Finally, funding will be required for: (1) development of novel field-based instrumentation; and (2) computational biology, including database construction, phylogenetic analyses, and computer modeling studies. The work outlined in this proposal is relevant to ongoing or proposed studies at Woods Hole, UC Boulder, Carnegie, Arizona, JPL, and Ames. The work will directly impact paleontological and geochemical efforts at Harvard, UCLA, and Arizona. We also anticipate development of field-based instrumentation at JPL and JSC. This concept is a very powerful, albeit expensive, initiative that provides a model for future collaborative investigations and interactions between different teams in the NAI. Since a project of this complexity level cannot be efficiently pursued by any single laboratory or team, this proposal offers the opportunity to establish a "path-finding" program that will have major impact, both within NASA's research agenda and the entire field of microbial biology and earth sciences.

This summary is just a start, and there are many details that require further study. They include: (1) assessing levels of interest by different teams in the NAI; and (2) determining required levels of support and reaching a consensus about identity of the most appropriate study site(s). The first step can be achieved via a discussion at the PI's meeting in December 1999 or during our next PI videoconference in January 2000. It would be desirable for NAI to organize and sponsor a small workshop in January 2000 to further explore the feasibility of modalities of such a program.

JUNE 2000 REPORT

After its introduction to the NAI in December, 1999, the Ecogenomics Focus Group held three videoconferences to discuss the formation of an NAI institute-wide project. The videoconference and associated e-mail interactions engaged investigators from each of the NAI teams and helped to clarify and enhance the objectives of our ecogenomics group.

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Through this process, we also recognized potential ties with a new proposal to form a focus group on evolutionary genomics. At least three of the teams submitted ecogenomics proposals to the NAI augmentation competition. Norman Pace at the University of Colorado submitted a proposal for studies of microbial diversity. Mitchell Sogin submitted a proposal that would closely link the MBL/Woods Hole ecogenomic efforts with corresponding activity at NASA Ames directed by David DesMarais and with Feran Garcia-Pichel at Arizona State University. In addition, Mitchell Sogin, David DesMarais, and Feran Garcia-Pichel (astrobiology investigators) submitted a multi-investigator grant with David Stahl of Northwestern University to the NSF (National Science Foundation) Biocomplexity competition. The NSF proposal would complement, but not duplicate, the research activities proposed for the NAI augmentation competition.

Required enabling technology includes: (1) microsensors for measuring biogeochemical parameters; (2) high-throughput DNA-sequencing facilities; and (3) DNA-microarraying facilities for monitoring gene expression. The impact of this work on understanding how ecosystems evolve may impact design of life detection experiments and will certainly play an important role in site selection for sample return missions. Finally, the information gained from these studies will allow us to design experiments that can evaluate the potential threat of sample return missions on Earth's microbial ecosystems.

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Appendix

Year 2

Year 2

Sidney Altman

Yale University

Philip W. Anderson

Princeton University

Sydney Brenner

Molecular Sciences Institute

Murray Gell-Mann

Santa Fe Institute

Ronald Greeley

Arizona State University

Robert B. Laughlin

Stanford University

Joshua Lederberg

The Rockefeller University

Elliott C. Levinthal

Stanford University

Richard J. Roberts

New England BioLabs

Anneila I. Sargent

California Institute of Technology

Maxine F. Singer

Carnegie Institution of Washington

The Roadmap is the product of efforts by more than 150 scientists and technologists, spanning a broad range of disciplines and organizations. More than 100 of these participated in a 3-day Roadmapping Workshop held in July 1998 at NASA Ames Research Center, while others attended previous topical workshops.

The Roadmap provides guidance for research and technology development across several NASA Enterprises: Space Science, Earth Science, and the Human Exploration and Development of Space. The recommendations were formulated in terms of 3 basic questions and 17 specific science objectives, which have been translated into NASA programs and integrated with NASA strategic planning.

HOW DOES LIFE BEGIN AND EVOLVE?

1. Sources of organics on Earth. Determine whether the atmosphere of the early Earth, hydrothermal systems or exogenous matter were significant sources of organic matter.
2. Origin of life's cellular components. Develop and test plausible pathways by which ancient counterparts of membrane systems, proteins and nucleic acids were synthesized from simpler precursors and assembled into protocells.
3. Models for life. Establish replicating, catalytic systems capable of evolution, and construct laboratory models of metabolism in primitive living systems.
4. Genomic clues to evolution. Expand and interpret the genomic database of a select group of key microorganisms in order to reveal the history and dynamics of evolution.
5. Linking planetary and biological evolution. Describe the sequences of causes and effects associated with the development of Earth's early biosphere and the global environment.
6. Microbial ecology. Define how ecophysiological processes structure microbial communities, influence their adaptation and evolution, and affect their detection on other planets.

DOES LIFE EXIST ELSEWHERE IN THE UNIVERSE?

7. The extremes of life. Identify the environmental limits for life by examining biological adaptations to extremes in environmental conditions.
8. Past and present life on Mars. Search for evidence of ancient climates, extinct life and potential habitats for extant life on Mars.
9. Life's precursors and habitats in the outer solar system. Determine the presence of life's chemical precursors and potential habitats for life in the outer solar system.
10. Natural migration of life. Understand the natural processes by which life can migrate from one world to another.

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11. Origin of habitable planets. Determine (theoretically and empirically) the ultimate outcome of the planet-forming process around other stars, especially as it relates to habitable planets.

12. Effects of climate and geology on habitability. Define climatological and geological effects upon the limits of habitable zones around the Sun and other stars to help define the frequency of habitable planets in the universe.

13. Extrasolar biomarkers. Define an array of astronomically detectable spectroscopic features that indicate habitable conditions and/or the presence of life on an extrasolar planet.

WHAT IS LIFE'S FUTURE ON EARTH AND BEYOND?

14. Ecosystem response to rapid environmental change. Determine the resilience of local and global ecosystems through their response to natural and human-induced disturbances.

15. Earth's future habitability. Model the future habitability of Earth by examining the interactions between the biosphere and the chemistry and radiation balance of the atmosphere.

16. Bringing life with us beyond Earth. Understand the human-directed processes by which life can evolve beyond Earth.

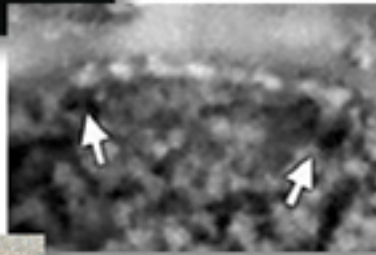
17. Planetary Protection. Refine planetary protection guidelines and develop planetary protection technology for human and robotic missions.

NASA Astrobiology Institute News Briefs

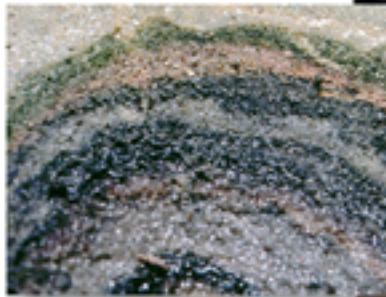
**"Apocalypse
Then"**



**"Scientists Find
Evidence of
Ancient Microbial
Life on Mars"**



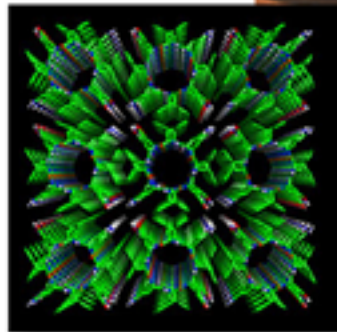
**"The Oldest
Life on Land"**



**"Two Rovers
in Search of
a Landing Site"**



**"One-Handed
Life"**



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